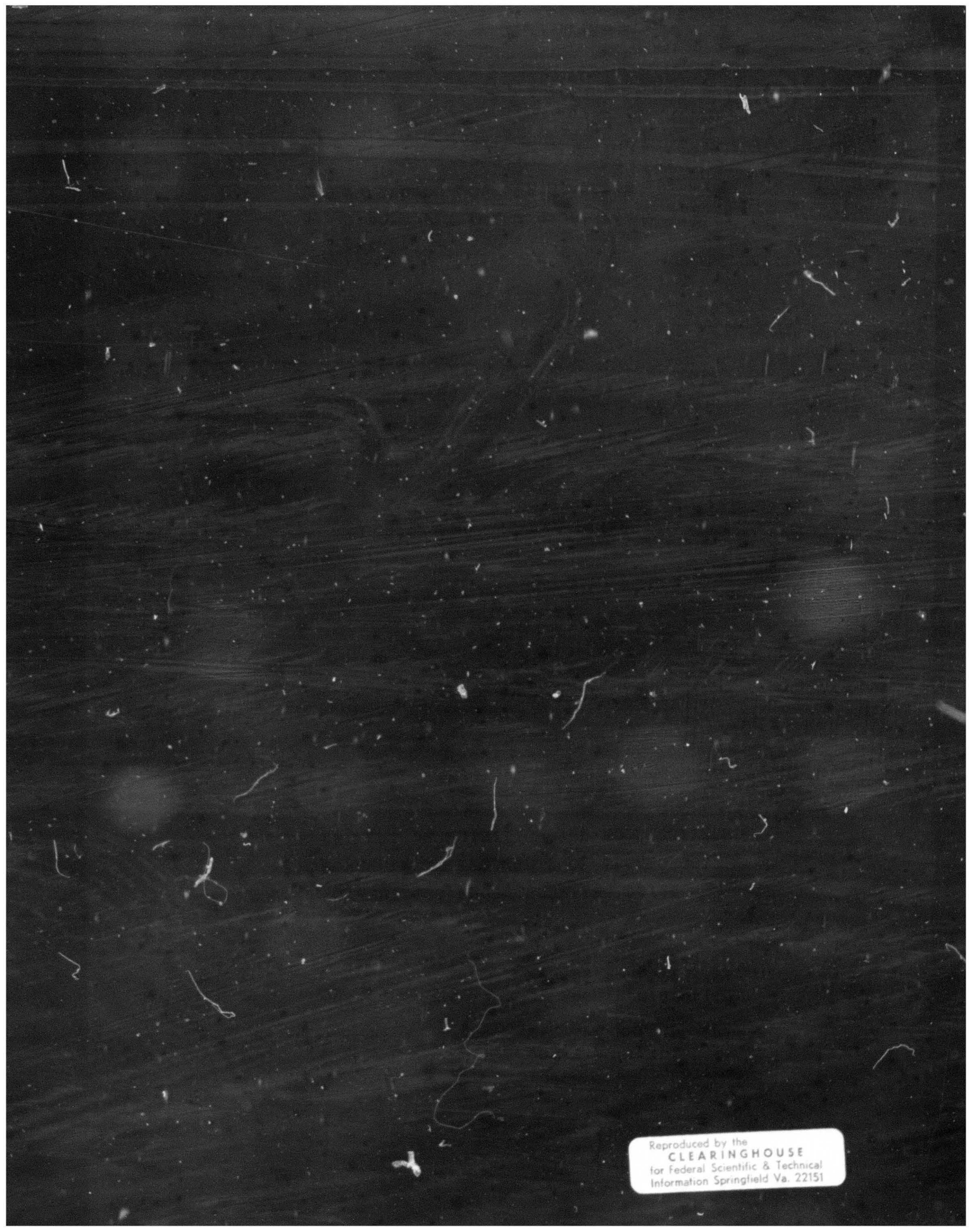

To: Aerospace Medical Division (AFSC)
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Attn of: SMSPP

MECHANISMS OF DETERIORATION AND FORMULATION OF SPACE DIETS

~~Project No. 41-609-00-46~~

Final Report

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SUMMARY

Model systems similar in composition to three types of freeze-dried foods were developed and used in studies on the effects of composition on the rates of deteriorative reactions at 55°C. Oxidation of lipids was found to proceed most rapidly at low water contents, while non-enzymatic browning and oxidation of proteins was more rapid at elevated water contents. Antioxidants and chelating agents had only a limited effectiveness which was also dependent on moisture content. During studies on dehydrated systems it was observed that hydrolysis of sucrose can occur even at low water contents and that the reducing sugars produced in the hydrolysis undergo considerable browning. Moisture contents corresponding to monolayer coverage by water were generally optimal in minimizing deteriorative changes.

Studies on actual foods were limited to a 3-week storage study at 55°C. At this relatively high temperature non-enzymatic browning was found to be the cause of deterioration. Under these conditions none of the foods were found to be satisfactory after storage, and oxidative changes were less important than browning. Consequently, the over-all quality of materials packaged under vacuum showed no improvement over that of materials packaged in air. Moisture content did have a significant effect, with high water contents resulting in more extensive deterioration.

INTRODUCTION

One of the major problems in applying dehydrated foods to civilian as well as military uses is the sensitivity of these foods to non-enzymatic changes such as lipid oxidation and non-enzymatic browning. Formulation of diets based on dehydrated foods to be stored under adverse environmental conditions is complicated by the following factors.

1) At low water contents lipid oxidation is greatly accelerated, but attempts to achieve protection from oxidation through elevation of moisture contents involve the risk of accelerating other reactions.

2) Different mechanisms of deterioration are interrelated, and the products of one reaction may affect the course of another.

3) Even when they are present only in trace concentrations, minor components of foods, such as metals, free amino acids, and others, exert an effect on deterioration.

This report describes studies on model systems representing key types of dehydrated foods and short-term studies on actual foods. These studies were designed to determine the effects of water activity and of composition on deteriorative reactions in dehydrated systems stored at 55°C.

STUDIES

ON

MODEL SYSTEMS

METHODS

Model System Composition

High fat-high protein model system. Based on the values for the various cuts of beef (Nutritional Data, 1956), the composition shown in Table 1 was chosen.

The lipid components added were varied to determine their effect on the over-all oxidation rate. Apiezon B, a highly saturated hydrocarbon, was used as the unoxidizable lipid component. Methyl oleate has a low susceptibility to oxidation; methyl linoleate is highly susceptible to oxidation.

The linoleate has a negligible ash content (less than 0.1 ppm of either iron, copper, or cobalt). The egg albumin has about 50 to 60 mg of ash per gram and after ashing of the model system the ash is about the same, indicating negligible ash contamination from the other components. The egg albumin contains, on the average, 0.35 µg of iron per gram, 1.25 µg of copper per gram, and negligible cobalt. The amount of water used was that necessary to form a uniform paste after mixing.

Low fat-high sugar model system. Based on the data available for apples (Nutritional Data, 1956), the general composition shown in Table 2 was chosen. The specific model systems used in Run 3 are shown in Table 3, and the systems used in Run 8 are shown in Table 4.

Potato starch model system. A model system was prepared (based on the composition of the potato) with the following ratios (in percent): potato starch, 45.5;

lipid, 10.0; protein, 4.6; and cellulose, 40.0.

The detailed compositions of the model systems used in the study, which were based on the above general composition, are shown in Tables 5 and 6.

Model System Preparation

The system was mixed by introducing the solids, such as the protein or sugar, into a 450-ml Sorvall Omni-Mix Cup modified with a screw port on the bottom, through which the sample may be extruded into reaction flasks. The lipid was then added drop-wise from a syringe concomitant to constant stirring with a glass rod to insure good mixing. The water, containing water-soluble additives such as the myoglobin, was then added. The blade section of the cup was inserted and the cup was attached to the mixer. An ice bath was put around the cup to keep the contents cool and the system was mixed for 10 min at maximum speed. The amount of water used was such that after mixing the system would resemble a slow-flowing paste which could easily be extruded from a cup. All model systems were prepared in a similar manner, with the lipid-soluble additives being mixed in with the lipid prior to addition of water-soluble additives as part of the water.

After mixing, a plunger was inserted into the cup and 2- to 5-g amounts were extruded through the bottom port directly into the reaction flasks. The flasks were tared prior to addition and weighed to 0.01 g after introduction of the wet model system. Specific flasks were used for the manometric studies, UV, peroxide value,

browning pigment, and head-space analysis. Immediately after weighing, the flasks were placed in a liquid nitrogen bath.

The frozen samples were then placed in a Vacudyne Pilot Plant Freeze-Dryer and dried for about 40 hr at 60 to 100 μ Hg and room temperature. The end point was determined to be when the vapor pressure rise of the chamber was less than 2 μ Hg per 30 sec. The vacuum was broken with pre-purified nitrogen and the samples were removed for the subsequent tests.

Isotherms

Isotherms were prepared at 55°C (+130°F) using the desiccator method. The constant humidity solutions used are presented in Table 7, in which the data has been extrapolated to 55°C (Rockland, 1960).

The samples were mixed as indicated above and then were extruded into 25-ml glass-stoppered weighing bottles. They were then freeze-dried and placed in duplicate into desiccators containing the constant humidity solutions shown in Table 7. After pulling a vacuum on the desiccators they were held at 55°C. After 2 days the samples were removed and weighed, then replaced. This operation was then repeated two more times.

Isotherms for the high fat-high protein and low fat-high sugar model systems are shown in Figure 1. A B.E.T. plot of the data between activities of 0.1 and 0.6 gave the results shown in Table 8.

The rate of attainment of equilibration in the desiccators was studied for the high fat-high protein model system. It is necessary to know this factor since, in the oxidation experiments, the samples must be pre-

humidified after removal from the freeze-drier. The results are shown in Figure 2 for 11 and 31% RH. It can be seen that the weight picked up in 1 hr exceeded the equilibrium value. This was probably due to the setting-up of a non-equilibrium situation during evacuation of the desiccator. During this time some pure water was boiled off and deposited on surfaces where it yielded an activity higher than that in the solution. In most preparations for deterioration studies a 3-hr equilibration period is used, since longer periods would lead to the occurrence of extensive reactions prior to controlled oxidation.

The isotherm for the potato starch model is shown in Figure 3 and the corresponding B.E.T. values are indicated.

Stability Studies

After preparation and removal from the freeze-drier, the respective samples were placed in desiccators maintained near dryness, at the monolayer, and above the monolayer. The samples were equilibrated for 3 hr at 55°C, removed by breaking the vacuum with air at the proper relative humidity, and stoppered or attached to their respective Warburg manometers. The samples were then analyzed by the following procedures during storage at 55°C.

Warburg oxygen absorption at 55°C. Duplicate Warburg manometers were used in a constant temperature water bath. A thermal barometer was used for each relative humidity. Apiezon B was used as the manometer fluid to insure measurement of very low rates of oxygen absorption, and

the data were calculated using the standard techniques, after correcting for sample volume.

Manometric data were plotted in the usual manner as oxygen absorbed vs. time. In addition, the square root of oxygen absorbed vs. time was plotted to illustrate the differences in rate constants.

Initially, it was assumed that the oxygen absorption would be due primarily to methyl linoleate and that the manometric measurements could be made using thermal barometer control flasks containing Apiezon oil and protein. This was found to be satisfactory for Run 1, in which the only lipid used was linoleate. In subsequent runs, however, in which the linoleate concentration was decreased, the amount of oxygen absorbed by the protein became significant with respect to total absorption because of the decreased absorption by the lipid component.

Furthermore, in contrast to lipid oxidation which, under most conditions, decreased with increasing water content, the amount of oxygen absorbed by protein increased with increasing moisture content. It became desirable, therefore, to study oxygen absorption by the protein in the absence of the lipid in order to be able to calculate separately the oxygen absorption by the protein and by the lipid. Two additional runs were made in which egg albumin alone was oxidized at several relative humidities including <0.1, 31, 40, 50, and 60% RH. The results are shown in Figure 4. The rates of oxidation by the protein were found to be constant with time, since the amount of oxygen absorbed increased linearly with time. Rates for each level of relative humidity were obtained by measuring the slopes of visually fitted straight lines. These rates

were found to be (in microliters of oxygen per [gram of protein x hours]): at 0.1% RH (dry), 0.353; at 31% RH, 0.389; and at the three highest humidities (40, 50, and 60% RH), 0.684. It is interesting to note that a sharp transition to a faster rate of oxidation by the protein occurs at the monolayer coverage humidity (31% RH). It appears that hydration of the protein increases its susceptibility to oxidation, possibly by increasing chain mobility and exposure of oxidizable side chains.

In order to use this data to calculate the oxygen absorption due to the lipid fraction the following analysis was used. The cumulative change in volume of gas space of the sample under study was measured 40 to 50 times during the course of a run. The empty thermal barometer flasks were used to correct external pressure and temperature fluctuations. The flask constant was calculated for each sample flask according to Equation 1.

$$\frac{\mu l\ O_2 \text{ absorbed (STP)}}{\Delta mm \text{ manometer reading}} = V_{O_2} = \frac{T_o}{T_1} \times \frac{1}{P_o} \times V \times 10^3 = 7 \times 10^{-2} V \qquad (1)$$

T_o = 273°K

T_1 = run temperature = 328°K

P_o = 1 atm of manometer fluid = 11,880

V = flask volume less the sample volume in cc

$$= V_f - \left[\frac{\text{weight of lipid}}{0.89} + \frac{\text{weight of protein}}{1.28}\right]$$

This flask constant was then multiplied by the corrected Warburg manometer reading for each time period to get the amount of oxygen absorbed over this period of time, as shown in Equation 2.

$$(\Delta V_{O_2})_{total} = \Delta mm \times 7 \times 10^{-2} \times V \qquad (2)$$

$(\Delta V_{O_2})_{total}$ = total oxygen (in microliters) absorbed during a given time interval

Δmm = change in manometer reading with respect to thermal barometer reading

The cumulative oxygen absorption at a given time was obtained by summing the oxygen absorbed during all of the preceding time intervals. The cumulative oxygen absorption was then divided by the dry sample weight to obtain the microliters of oxygen absorbed per gram of sample. This was then presented graphically as microliters oxygen per gram of sample vs. time.

The oxygen absorbed by the protein was then calculated using the rates derived from the results shown in Figure 4. To obtain the amount absorbed by protein at 0.1% RH (dry), for instance, Equation 3 was used.

$$\sum_{o}^{t}(V_{O_2})_{protein} = (0.353)(t)(W_{protein}) \qquad (3)$$

$(V_{O_2})_{protein}$ = microliters oxygen absorbed by protein

t = time in hours

$W_{protein}$ = weight in grams of the protein in the sample

This value was then subtracted from the cumulative total oxygen absorbed at each value of time to give the amount

of oxygen absorbed by the lipid. Assuming that methyl linoleate is the only lipid component absorbing oxygen, oxygen absorption by the linoleate was calculated using Equation 4.

$$\frac{\mu l}{g} = \frac{\sum_{o}^{t}(V_{O_2})_{total} - \sum_{o}^{t}(V_{O_2})_{protein}}{W_{lin}} \qquad (4)$$

W_{lin} = weight in grams of the linoleate in the sample

In order to simplify these calculations, a modified Fortran-FAP computer program was written and the results were calculated using a DEC-PDP-8S digital computer. This program was also set up to calculate other derived parameters characterizing the kinetics of oxidation.

Square root plots. As was shown in the first quarterly report, under certain conditions a plot of the square root of the oxygen absorbed vs. time gives a straight line whose slope is proportional to the rate of oxidation. This occurs in the early stages of oxidation when the rate is proportional to the monomolecular decomposition of the formed hydroperoxides. Under these conditions the rate of oxidation is

$$-\frac{d(O_2)}{dt} = \frac{d(ROOH)}{dt} = k(M)^{\frac{1}{2}}(RH)(ROOH)^{\frac{1}{2}} \qquad (5)$$

O_2 = oxygen absorbed
ROOH = hydroperoxide content
M = trace metal catalyst content

RH = substrate concentration
k = rate constant

When catalyst inactivation and substrate depletion are assumed to be small the over-all monomolecular rate constant Km is

$$Km = k(M)^{\frac{1}{2}}(RH) \tag{6}$$

Under these conditions, integrating the first equation gives

$$(O_2)^{\frac{1}{2}} = (ROOH)^{\frac{1}{2}} = \frac{Km}{2}t \tag{7}$$

Thus, a plot of the square root of oxygen absorbed vs. time should give a straight line. It has also been found that the magnitude of Km is a function of the moisture content (Maloney *et al.*, 1966) with a decreasing value as the equilibrium humidity is increased. This has been explained as being due both to inactivation of the trace metals present and to hydrogen bonding of hydroperoxides with water to prevent them from further reaction.

Initial extent of oxidation. The initial extent of oxidation was measured by extracting a sample at each humidity with a known amount of chloroform:methanol (3:1), filtering the solution, and measuring the optical density at 233 mμ on a Hitachi-Perkin-Elmer Spectrophotometer. The extent of oxidation was then calculated from the following formula.

$$\frac{\text{moles oxygen absorbed}}{\text{moles oxidizable lipid*}} = \frac{\dfrac{OD(233)}{\text{mg lipid per cc}} - 0.088}{79.64} \tag{8}$$

* Methyl linoleate.

In the cases where Apiezon B was used as part of the lipid phase, the initial extent of oxidation could not be determined by UV analysis because of the high chromophore content of the oil. In these cases the initial oxidation was assumed to be negligibly small.

Lipid extraction. For each model system prepared, duplicate samples were extracted with a suitable solvent system in order to determine the lipid content. This figure was then used to determine the lipid content of all other samples prepared in that batch. For the model systems described above the method was the following.

Eighty cubic centimeters of chloroform:methanol (3:1) were added to a sample which was then shaken for 1 hr on a rotary shaker. It was then filtered through a Buchner funnel into a pre-tared 24/40 150-ml flask to remove the solids and the filter cake was washed with additional solvent. After removing the solvent on a rotary evaporator (50°C, 30" Hg, 1 hr), the remaining lipid was weighed. The average extraction value was used for all subsequent calculations.

Moisture content. Duplicate samples from each desiccator were used for moisture determination by measurement of the equilibrium vapor pressure, using either a high sensitivity manometric system or a sensitive method based on gas chromatography, as described below.

Twenty milliliters of dry methanol were added to the sample in a 50-ml stoppered Erlenmeyer flask and shaken for ½ hr. A suitable sample (500 to 1000 µl) was taken with a syringe and injected into a Perkin-Elmer Gas Chromatograph equipped with a thermal conductivity detector at 100°C. The column was 6' x ¼" copper

tubing packed with Poropak Q and the gas was helium at 40 cc per min. A standard was made by adding 1 cc water to 49 ml of the methanol used for the determination. Standard techniques were used for measurement of peak areas.

Browning pigments. The method for determination of the non-enzymatic browning pigments formed is presented below (modified from Choi *et al.*, 1949).

Two grams of dry material were dispersed in 20 cc of distilled water. Two and five-tenths milliliters of a 10% trypsin suspension were then added. (The trypsin suspension must be prepared just minutes before each analysis.) After incubation at 45°C for 1 hr, 2 ml 50% TCA and 0.1 g Celite (analytical filter aid) were added. After mixing and filtering, the percent transmission at 400 mμ was measured, setting the enzyme blank at 100% T.

The results were reported as (OD per gram of sample) x 100 in order to put all samples on an equal basis for comparison.

The trypsin procedure described above was found to be unnecessary in some runs, in which the 2 g of dry material were extracted only with 10 ml of water plus 10 ml of methanol and the optical density was measured at 400 mμ.

Fluorescent browning pigments. The material was extracted in the same manner as was used for the optical density measurement of browning pigments. From this solution 200 μl were spotted on a cellulose thin-layer plate and dried. The spot was then eluted with propanol: water (95:5, v/v) and fluorescence was detected by observing the plate in a dark room under a short-wave UV lamp.

Reducing sugars. The dried model system was extracted with 25 ml water for ½ hr and filtered under vacuum to remove the insoluble components. Five milliliters of solution were removed and diluted to 20 ml in a 125-ml flask. To this solution 10 ml of Reagent A* were added along with a boilezer. This was then heated over a flame, brought to boiling in exactly 2 min, and then boiled for an additional 5 min. The flask was cooled rapidly under cold tap water and 10 ml each of Reagents B and C* were added. The solution was then titrated with 0.1 N sodium thiosulfate using starch indicator for the end point. A water blank was used as the correction factor and a calibration curve was prepared with equal amounts of glucose and fructose. On this basis 1 meq of thiosulfate (meq blank minus meq sample) equals 33 mg of reducing sugar as glucose. The percent hydrolysis is then the amount of glucose determined per milliliter divided by the amount of sucrose originally present times 1.9 to convert to a molar basis.

Glucose oxidase procedure. The *Glucostat* procedure of the Worthington Biochemical Corporation was employed in Run 3. In this test glucose oxidase reacts with the glucose present to form hydrogen peroxide. This in turn oxidizes a chromogen dye which can be measured at 400 mμ. This test was not used for Run 8 since other data (Schobell, T., Tannenbaum, S. R., and Labuza, T. P. Unpublished data, M.I.T., 1967) indicate that it is unreliable for supersaturated sugar systems such as the dry model.

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* Reagent A: $CuSO_4$, 30 g; Na_2PO_4, 90 g; KNa tartrate, 225 g; KIO_3, 1.75 g; water to 1 liter.
Reagent B: KI, 90 g; koxalate, 90 g; water to 1 liter.
Reagent C: H_2SO_4, 59 ml; water to 1 liter.

*Procedure for peroxide.** For dried samples containing between 2.5 and 5.0 g of material, the sample was shaken with 60 cc of a mixture of chloroform:methanol (3:1) for 10 min, then filtered into glass-stoppered flasks. The solvent was evaporated in a flash evaporator, breaking with nitrogen at the end of the process. The lipid remaining in the flask was weighed and 10 ml of a mixture of glacial acetic acid:chloroform (3:2) was then added and dissolved. Five-tenths of a milliliter of potassium iodide (saturated solution) was added. (This solution must be prepared just minutes before each analysis.) *Exactly 2 min* after the addition of the potassium iodide 10 ml of distilled water were added and the liberated iodine was titrated with 0.01 N sodium thiosulfate solution. To observe the end point 1 ml 5% starch solution must be added to the system before the titration. The end point is indicated by disappearance of the blue color.

Calculation in milliequivalents per kilogram of lipid:

$$PV = \frac{\text{normality } x \text{ 1000 } x \text{ ml } Na_2S_2O_3}{\text{weight of lipid}} \qquad (9)$$

To convert to microliters oxygen absorbed per gram of methyl linoleate the peroxide value is multiplied by 11.2.

In the case of high sugar systems containing oil, extraction procedures normally used in preparation for peroxide tests could not be used, and the peroxides were estimated using a thin-layer chromatography procedure described below.

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* Modified from the A.O.C.S. method.

One-millimeter thick Silica Gel-G plates were prepared and dried at 60°C for 1 hr. Twenty cubic centimeters of methanol were added to the sample to be studied in a 50-ml Erlenmeyer flask and shaken for 1 hr. Five microliters of the sample suspension in methanol were then spotted on the plate, and the components separated using a 95:5 hexane-methanol liquid phase mixture. When the solvent front was near the top of the plate, the plate was dried at 60°C, and then the spots were visualized by iodine vapor and subsequently permanently fixed in a sulfuric acid spray. In this system the unoxidized methyl esters move to the top of the solvent front, peroxides to about 1/3 of that distance, and more polar compounds stay at the origin.

Metal analyses. The ashing technique and procedures for: iron using 1-10-o-phenanthroline; copper using sodium diethyl dithiocarbamate; and cobalt using Nitroso-R-salt were standard techniques taken from Sandell (1950).

Amino acid degradation. In Run 5 histidine was added to the model system in order to study its effect on oxidation rates. In order to measure degradation of the histidine during the oxidation, a thin-layer chromatographic procedure similar to that for peroxide determination was used, with the following modifications. The solvent system for developing the plates was butanol:acetone:water (25:6:25) and the plates were sprayed with a solution of 300 mg 1,2,3-triketohydrindene hydrate per 100 ml butanol: acetone (97:3).

Head-space analysis. A gas chromatographic procedure (Karel *et al.*, 1963) was used for the determination of oxygen consumption and carbon dioxide production.

RESULTS AND DISCUSSION

High Fat-High Protein Model Systems

Run 1. The initial stability test performed was on the high fat-high protein model system. In this case the lipid consisted entirely of twice-distilled methyl linoleate having an initial extent of oxidation of 0.536% (410 µl oxygen per gram). A single model system was made which, after freeze-drying, was equilibrated to 0, 11, and 31% RH.

The Warburg manometer samples were run in duplicate, and the over-all results for oxygen absorbed are presented in Figure 5. As shown in this figure, the rates of oxidation were very rapid and greatly exceeded those previously observed in a cellulose-linoleate system. This indicates that myoglobin is a very effective catalyst even though the amount of iron added by its addition is less than 0.2 ppm. The protein surface may also be favorable to a more rapid rate.

Also, it can be seen that the humidification did significantly reduce the oxidation rate. This was similar to the results found by Maloney *et al.* (1966) for cellulose model systems. The effect of humidification can also be illustrated by plotting the square root of oxygen absorbed vs. time. In this type of plot the slope is proportional to the over-all rate constant during the initial, monomolecular phase of oxidation. Figure 6 shows that the slopes for the humidified samples are slightly less; the break point from the initial straight line position occurs at a higher value for the 11% RH sample than for the dry

sample and even extends off the figure for the 31% RH sample. This indicates that the monomolecular period is prolonged. These results support the work of Maloney *et al.* (1966) and Labuza *et al.* (1966), in which it was found that a similar situation existed for cellulose model systems. The results were explained by the hypothesis that the formed hydroperoxides were being tied up by hydrogen bonding at the water-lipid interfaces and that the catalysts present were partially deactivated by hydration with water.

The manometric results were not carried any further because the rates became too rapid for accurate measurements. The UV value used to calculate the initial extent of oxidation is shown in Table 9. This calculation was performed on the samples after removal from the desiccators. The values are lower, for all samples, than that of the methyl linoleate prior to preparation of the model system. This may indicate either that a portion of the hydroperoxides were tied to the protein and became unextractable or that the protein surface catalyzed their decomposition during the preparative procedure.

The data for peroxide value are also shown in Table 9. The initial oxidation values are about 1½ to 2 times higher than those found by UV, which may be due to the fact that the samples had to be held in the freezer for 12 hr before the analysis could be performed. The results of peroxide tests as a function of moisture content showed the same effect as was shown by the manometric data at a higher level of oxidation, and they indicate that the method may be useful as an index of deterioration in this study.

The results of oxygen concentration in the head space

(also presented in Table 9) were similar to those which were observed for peroxide values and from the manometers. The dry and 11% RH samples oxidized most rapidly and the 31% RH sample showed an inhibited rate of oxidation which is an indication of the protective effect of water.

The results of tests related to non-enzymatic browning are shown in Table 10. Although the system contained no reducing sugars, the production of both brown color and carbon dioxide indicates that some browning took place. This may be due to reactions of the aldehydes and ketones which are formed as products of oxidation and can react with the protein in the presence of water. The results also show a faster rate in the highest humidity sample.

Run 2. Three model systems were prepared; their compositions are described in Table 11. Each was divided into three lots and humidified to 0, 31, and 50% RH, values corresponding to water contents respectively below, at, and above the monolayer.

The antioxidants were added as follows.

Butylated hydroxy toluene - One-tenth gram was dissolved in 10 g Apiezon B oil. Five grams of this solution were added as part of the lipid to the system, giving a concentration of 1.6×10^{-3} moles per mole of linoleate.

Propyl gallate - One-tenth gram was dissolved in 100 ml water. Five milliliters of this solution were added as part of the water to give a concentration of 1.65×10^{-3} moles per mole of linoleate.

Butylated hydroxy toluene is a lipid-soluble antioxidant. Propyl gallate is a water-soluble antioxidant.

Both were added at a level which has been found to be effective in dry cellulosic model systems (Karel *et al.*, 1966), and this concentration is at the maximum allowable level prescribed by the United States Food and Drug Administration (50 ppm on a dry weight basis for dried cereals).

Total oxygen absorption is shown in Figures 7, 8, and 9. The rate of oxygen absorption is relatively low, probably because of the low concentration of linoleate in the system. As would be expected, oxygen absorption by the protein increases with increasing relative humidity. When this data is considered in analyzing the total oxygen absorption (Figures 7, 8, and 9) it is evident that in Run 2, in which linoleate concentration was low, up to 80% of the oxygen was absorbed by the protein.

The effects of antioxidants and of relative humidity on the lipid component oxidation are evident from the data presented in Table 12, in which the calculated oxygen absorption attributable to the linoleate is presented for the different experimental conditions. The following effects were seen.

Humidification to 31% RH substantially reduced the oxygen absorption by the control, whereas at 50% RH the absorption was increased. The effect at 50% RH was unexpected and may have been due to interactions with protein oxidation products or to increased mobility of some prooxidants.

Propyl gallate was an effective antioxidant, especially at high humidities.

Butylated hydroxy toluene showed antioxidant activity, with a less synergistic effect with water, than was apparently the case with propyl gallate. With butylated

hydroxy toluene, too, an initial prooxidant effect was observed, as is frequently the case with some antioxidants.

Table 13 presents the peroxide value measurements for Run 2, expressed as milliequivalents of oxygen absorbed per kilogram of linoleate. On the basis of total lipid the values should be divided by 10. As in the case of food systems the values show no clear trend of an increase with time in proportion to the amount of oxygen absorbed. For cellulosic model systems a direct correlation of peroxide value to oxygen absorption has been found (Martinez and Labuza, 1966). For each moisture content the peroxide value of the control sample increased to a maximum and then decreased. The butylated hydroxy toluene samples at 31 and 50% RH began at a high value and then decreased whereas the dry sample varied extensively. The propyl gallate samples did not vary extensively. It seems reasonable that, at least for this system where the total amount of lipid oxidation is small, measurement of peroxide value is not a good indicator for comparison of the various treatments.

Table 14 presents the data for non-enzymatic browning reactions as measured by carbon dioxide and brown pigment production. The small amount of carbon dioxide produced and the negligible increase in color show that for this type of system, in which oxidation is very slow, the extent of non-enzymatic browning is small even at the higher moisture contents.

Run 4. The conditions of Run 4 (compositions described in Table 15) differed from those in Run 2 primarily in two respects.

1) Concentration of linoleate was increased to 30% of the total lipid.

2) Cobalt nitrate was used as the catalyst at a concentration higher than that used with myoglobin (about 100 times greater concentration of metal on a molar basis).

Total oxygen absorption data are presented in Figures 10, 11, and 12. As one would expect on the basis of the conditions described above, the rate of oxidation is much greater than that in Run 2, and the percentage of the absorption attributable to the protein is much less (10 to 20% of the total). The shape of the absorption curves in this run is more typical of the lipid oxidation chain reaction than in Run 2, in which protein oxidation dominated the kinetic behavior. The data on oxygen absorption attributable to the linoleate are presented in Table 16. The following effects of composition and humidification on lipid oxidation were evident.

Humidification had a substantial antioxidant effect, probably due to inactivation of the catalyst. Humidification to the monolayer value (31% RH) appeared more effective in this respect than humidification to a higher level.

Propyl gallate was effective at all humidities, but it was most effective at the monolayer value (31% RH).

Butylated hydroxy toluene showed an effect which was similar in all respects to that of propyl gallate.

The major difference between Runs 2 and 4 with respect to the behavior of the antioxidants was the following. In Run 2 propyl gallate was very much more effective than butylated hydroxy toluene when the samples were oxidized at increased humidities, whereas in Run 4 both

were equally effective. Whether this difference was due to differences in the catalyst used or to the difference in linoleate concentration is at present unknown. The synergistic effect of humidification and antioxidant, however, was observed under all conditions.

The progress of oxidation was also followed by two additional techniques: oxygen analysis in head space of reaction flasks (Table 17) and peroxide value determinations (Table 18). The head-space analysis was too variable to allow specific conclusions. The peroxide values, however, confirmed the manometric data discussed previously.

As was discussed in previous reports, measurements of optical density of aqueous extracts from the system and of carbon dioxide concentration in the head space give information on progress of non-enzymatic browning. The data for Run 4 are presented in Tables 19 and 20. In the systems studied in Run 4 browning did not appear to be very significant, as evidenced by the lack of significant increases in either optical density or carbon dioxide. In the case of samples containing propyl gallate, some carbon dioxide appears to have been produced during the steps preceding oxidation experiments, but there is no significant carbon dioxide evolution during the oxidation experiment.

Run 5. In Run 5 the effects of the cobalt catalyst, of the amino acid histidine, and of the metal chelating agent EDTA were studied. In contrast to previous runs, control samples containing no catalyst were used. The compositions of the systems are shown in Table 21. The experiment was designed to study the progress of oxidation at 0% RH, at the monolayer value for water (31% RH), and

at a humidity above the monolayer value (40% RH). Due to mechanical failure in the oxidation bath, no manometric oxygen absorption data could be obtained for the dry system. The data for the other two humidity levels are shown in Figures 13, 14, 15, 16, 17, and 18. These figures present the total oxygen absorption, which was substantially higher than in Run 4 due to the increased concentration of linoleate in the lipid phase (40%). The contribution of oxygen absorption by protein to the total oxygen absorption was less than 10%. The oxygen absorption attributable to the linoleate oxidation is shown in Table 22. The following effects were observed.

In the absence of added cobalt, increase in humidification to above the monolayer value decreased the rate of oxidation. This might be expected if hydrogen bonding between peroxides and water is the predominant mechanism of water activity.

Samples containing cobalt as the only additive showed results similar to those seen in Run 4, with 31% RH more inhibitory to oxidation than 40% RH. At 31% RH the prooxidant effect of cobalt appears to be completely eliminated.

Histidine alone or in combination with cobalt was very effective in reducing the rate of oxidation at 31% RH. At 40% RH cobalt plus histidine had some antioxidant activity, but histidine alone was ineffective.

EDTA alone showed effects similar to those observed with histidine alone, possibly indicating that both act as trace metal chelators.

EDTA in the presence of cobalt reduced the prooxidant activity of the cobalt, but this combination did not show the very strong antioxidant activity observed with the

cobalt-histidine system.

Some data on peroxide values were obtained and are presented in Table 23. The number of samples was too small to allow quantitative analysis, but certain conclusions based on the manometric data appeared to be confirmed by the peroxide values.

Thin-layer chromatography showed development of peroxides in all the samples. Thin-layer chromatography of the histidine samples showed no observable spots. This was due either to complete histidine degradation or to the fact that histidine may have been tied up by the lipid or protein fraction.

Low Fat-High Sugar Model Systems

Run 3. Two model systems were prepared and are described in Table 3. A third system of 100% glucose was also prepared. However, due to the high adsorption of water by glucose, a glassy hard system formed which became impossible to extrude in the normal manner.

As mentioned previously, because extraction of the lipid in these systems was difficult oxidation was measured by thin-layer chromatography for presence of hydroperoxides. Samples from each system were taken at 0, 4, 7, 13, and 29 days. No spots other than methyl oleate were present, indicating that oxidation may not be an important factor in these systems.

The extent of browning as a function of time is presented in Figure 19 for the 100% sucrose model system and in Figure 20 for the 50:50 glucose-sucrose system. Theoretically, no browning should take place if a non-reducing sugar such as sucrose is used, but, as can be

seen, there was a definite rate of color increase. This indicates that reducing type groups were available either from the protein, from oxidation of the lipid (shown above to be negligible), or from hydrolysis of sucrose. In order to determine whether sucrose hydrolysis had actually taken place the following tests were made on the model systems.

Reducing sugar test. If all the reducing sugar estimated by this test came from hydrolysis of sucrose, one-half of it could be expressed as glucose and is so reported in Table 24. It should be noted, however, that this test may also estimate other reducing groups such, for instance, as may be present after subsequent browning of any liberated glucose or fructose.

Glucose oxidase test. This test is an enzyme-based test specific for glucose only. The results tabulated in Table 24 show that some hydrolysis of sucrose occurred in the model systems. Furthermore, it shows that at high moisture contents the amount of reducing sugars was greater than would be expected on the basis of the glucose oxidase test. This result suggests that a significant part of the hydrolysis products of sucrose underwent further reactions, probably due to non-enzymatic browning.

These results show the importance of moisture in controlling non-enzymatic browning and hydrolysis on non-reactive sugars.

The results of head-space analysis are presented in Table 25. Although extremely variable, they seem to coincide with the browning data.

Run 8. In Run 8 the effects of composition were studied on the stability of a high sugar system simulating products such as orange juice powder or apple sauce. The compositions of the three systems tested are presented in Table 4. This case differed from Run 3 in that no lipid was used and sucrose was the only carbohydrate present. Table 4 also presents the moisture contents at the three relative humidities used.

The extent of browning can be seen in Figures 21, 22, and 23, respectively, for each system. It is obvious that sucrose alone (without any acid being present) does not brown. As shown in Figure 22, in the presence of acid significant browning occurred even at 0% RH, which is well below the monolayer. The protein added to the system (Figure 23) seemed to act as a buffer. It completely prevented browning at 0% RH and slowed it down at 31 and 75% RH to below that of the dry samples without protein. This phenomenon may have been due either to the buffering action of the protein or to its water-binding capacity.

As was seen in Run 3, sucrose hydrolysis occurred, producing the necessary reducing groups for browning; this occurred even at the low relative humidities. In Table 26 the results of sucrose hydrolysis for Run 8 are shown. As expected, they seem to parallel the browning data. In addition, even in the dry state in System II (Table 4) appreciable hydrolysis can be seen. It is obvious from these data that the dehydrated system was not entirely inert and that the water present allowed acid hydrolysis followed by browning to occur. Table 27 contains the data for the carbon dioxide product, which also confirms the above conclusion.

Run 10. In Run 10 the hydrolysis of sucrose was studied at 25°C in a completely saturated solution to learn the effect of hydrogen ions on the rate. The system used was comprised of (in grams): sucrose, 211; water, 100; and citric acid, 5 or 10. Hydrolysis was measured by both reducing sugar and glucose oxidase tests.

The data are presented in Figure 24 as a function of time and are replotted as a first-order kinetic plot in Figure 25.

The samples containing 5 g of citric acid had an initial pH of 1.69; this decreased to about 1.59 after 3 weeks. The first-order rate constant was 2.4×10^{-3} hr^{-1}. For the 10-g citric acid samples the initial pH was 1.55; it had decreased to about 1.38 after 3 weeks. This system had a first-order rate constant of 3.5×10^{-3} hr^{-1}. In neither case did browning occur in any of the samples.

From these tests it can be concluded that:

1) pH was very important in controlling the rate of hydrolysis of sugar in foods under storage.

2) When enough water was available hydrolysis occurred but browning did not. This may have been due to dilution in the large amount of water. However, in dried foods hydrolysis will lead to excessive browning.

Potato Starch Model Systems

Run 6. In Run 6 the effects of moisture content and of two antioxidants were measured in a model system based on the composition of potatoes. The compositions of the models tested are presented in Table 5. In each case,

methyl linoleate was used as 100% of the lipid.

Figures 26, 27, and 28 present the oxygen absorption per gram of sample as a function of relative humidity. Oxygen absorption attributable to linoleate is presented in Table 28. The following effects were observed.

The rate of oxidation in the dry state as compared with high protein models was very high.

Humidification to 11% RH was much less effective than humidification to a level above the monolayer (31% RH).

Both antioxidants had only a small effect on the overall rate of oxidation, although they were used at the same level as in the high protein-high lipid model systems (10^{-3} moles per mole of linoleate). Butylated hydroxy toluene had a slight inhibitory effect in the dry state but was not as effective at 11% RH. In fact, in some cases butylated hydroxy toluene samples oxidized so rapidly even at the higher moisture contents that no data could be obtained. Since butylated hydroxy toluene is volatile it may be lost during freeze-drying. However, the increased rates were even faster than the controls, indicating some secondary mechanism. Because of this lack of reproducibility between butylated hydroxy toluene samples it is probably not a good antioxidant to use. Some data on peroxide values were obtained and are presented in Table 29. Compared to those of previous model systems the values are very high; this indicates a more favorable environment. In the high protein model systems the hydroperoxides probably react very readily with the protein and thus their level is low. The data in Table 29 confirm the manometric oxidation data showing an inhibitory effect with increasing moisture content. In addition, at the higher moisture contents the peroxide

values for the propyl gallate systems were much lower than those for the controls; this might indicate a significant antioxidant effect based on peroxide value. However, the data in Figures 26, 27, and 28 show that this was not the case. The explanation for this is that the action of propyl gallate is to decompose the hydroperoxides formed; thus, the over-all magnitude of oxidation may be the same with a much lower peroxide value. Data for browning showed no increase and are not reported.

Run 7. In Run 7 a model system based on potato starch and having the composition presented in Table 6 was studied. The major differences from Run 6 were that linoleate was used as only 30% of the lipid and that an additional system containing 5 ppm cobalt (based on linoleate) was tested.

The data for total oxygen absorbed per gram of dry sample are presented in Figures 29, 30, and 31. The data for oxygen absorbed per gram of linoleate are presented in Tables 30, 31, and 32 for duplicate samples at each relative humidity. From these data the following conclusions can be drawn.

The total rate of oxidation was much slower than that in Run 6 due to the decrease in the linoleate concentration.

Humidification to 11% RH significantly decreased the rate of oxidation of the samples, especially in the cobalt-catalyzed samples.

Humidification to 31% RH, although decreasing the rate in relation to the dry samples, increased the rates of all samples relative to those at 11% RH. This has also been found previously for the high

protein model systems.

The antioxidant butylated hydroxy toluene acted as a distinct oxidation catalyst, and as in Run 6, duplicate samples showed poor correlation. These results indicate that butylated hydroxy toluene should not be used as an antioxidant. Propyl gallate as an additive was not an effective antioxidant.

The peroxide values are presented in Table 33, but no correlation can be made with the oxygen absorption data. As in Run 6, browning was not significant and is not reported.

In order to predict the extent of oxidation as a function of time, the oxidation data (on the basis of linoleate) for Runs 6 and 7 were plotted as square root plots (as described in the METHODS section) and examples are given in Figures 32 and 33, respectively. The monomolecular rate constant Km for each system is shown in Table 34. As observed previously, both the effect of humidification and the prooxidant effect shown by the antioxidants are confirmed. The reduction in rate with humidification was probably due to catalyst inactivation. The increase in rate at 31% RH may have been due either to promotion of surface mobility of the free radicals or to release of new catalysts by swelling of the polymer chains.

SHORT-TERM

STORAGE STUDY

A short-term accelerated storage study was made on three selected freeze-dried foods: ground roast beef, diced potato, and prepared applesauce. These foods were chosen because they were closest to the model systems studied and direct comparisons could be made. The foods were freeze-dried, humidified at three different levels, and stored under various oxygen partial pressures at 55°C for 3 weeks. Storage deterioration was measured on the basis of the tests found applicable in the model system studies. The results of the tests on both the model systems and the foods were correlated so that a prediction of storage stability for future foods could be made.

Materials

Beef. A 7-lb piece of fresh USDA Choice top round was cut and trimmed of all visible fat and gristle, ground in a silent cutter, and cooked in its own juices in molds at 10 lbs steam for 1½ hr. The yield of 3½ lbs was divided and to one-half of it 1 mg of propyl gallate and 1 mg of histidine dissolved in 25 cc water were added and mixed 1 min on the silent cutter. The control sample was mixed in the same manner with water. The ground beef was then spread on trays, frozen at -40°F, and freeze-dried at 50 μ Hg for 4 days at room temperature. The addition of histidine and propyl gallate gave approximately 25 ppm of each on the basis of the fat content of the beef.

Potato. Ten pounds of Maine potatoes were washed, peeled, and diced into 3/8-inch cubes; the yield was 6 lbs of potato. This was spread on a grating tray and

cooked for 20 min in flowing steam, cooled at room temperature, then frozen at -40°F. The freeze-drying procedure was the same as that used for the beef.

Applesauce. "Motts" applesauce (5 lbs, 5 oz) was mixed and spread on trays, frozen at -40°F, and freeze-dried for 7 days at 80°F and 75 μ Hg.

Humidification

After freeze-drying, the vacuum was broken with nitrogen and the dried product was divided into thirds and humidified (in the manner used for the model systems) to values of 0, 31, and 50% RH. Equilibration was approximately 4 hr. The moisture contents and B.E.T. values are presented in Table 35.

Packaging

The humidified product was then weighed into foil pouches comprised of 0.5 mil mylar, 0.3 mil aluminum foil, and 3.0 mil high density polyethylene. The amounts used were (in grams; ± 0.1 g): meat control, 2 g; meat plus propyl gallate plus histidine, 2 g; potato, 3 g; and applesauce, 9 g. The pouches were then sealed on a Flex-Vac vacuum pouch sealer at a heat setting of 550°F under the following three atmospheric conditions.

Meat systems.

Air pack: normal air.

Middle pack: package blown with 90:10 (v/v) nitrogen to oxygen prior to sealing at 5" to

20" vacuum.

Vacuum pack: greater than 29" vacuum.

Potato.

Air pack: normal air.

Middle pack: blown with air prior to sealing at 5" to 20" vacuum.

Vacuum pack: 29" vacuum.

Applesauce.

Air pack: normal air.

Vacuum pack: 29" vacuum.

Storage

Individual pouches were prepared for each of the following tests: browning, peroxide value, head-space analysis, organoleptic analysis, moisture content, and fat content.

The samples for moisture content and fat content were frozen at -40°F until used. Initial values were determined immediately for all tests except the organoleptic analysis, for which the samples were stored at -40°F until the end so that comparison with the samples stored at 55°C could be made. The other pouches were then stored at 55°C and tests were made after 10 days and 3 weeks.

Specific Tests

Head space. Head space in the foil pouches was determined by puncturing the pouch with a needle attached to a gas burette and transferring the gas content to the

burette. This was easily accomplished with the air and middle packs but no sample could be obtained from the vacuum pack because of the extremely low vacuum. A 1-ml sample of the gas was then analyzed for carbon dioxide, oxygen, and nitrogen in the usual manner.

Package vacuum. To measure vacuum in the middle packs, each was placed in a vacuum desiccator to which a gauge had been attached. A vacuum was then pulled on the desiccator at about 5" per min. The package vacuum was recorded at that reading at which the package began to flex.

For the vacuum pack pouches, vacuum was always greater than 29" Hg and was not recorded separately.

Browning tests. For the meat systems, the trypsin test, as described previously, was used.

The test for potato was the same as that for Runs 6 and 7; however, 30 ml of water were used.

For applesauce, the same procedure was used as for Runs 6 and 7. However, due to the gelling nature of the material 20 ml of water plus 10 ml of methanol were used.

Organoleptic tests. The samples were rated for flavor, color, and odor on a nine-point hedonic scale (1 ⟶ 9 as poor ⟶ best) by four members of the laboratory.

Peroxide value. The procedures were the same as those used for the model systems.

RESULTS

Run 9 was set up as a short-term accelerated storage

test of three actual freeze-dried foods whose compositions would be similar to those of the model systems.

Model System	*Freeze-dried Food*
High fat-high protein	Ground beef (~20% lipid on a dry weight basis)
Low fat-high sugar	Applesauce (~90% sugar)
Low fat-starch	Potato

The foods were prepared as described above and were stored for 3 weeks at 55°C under various moisture and oxygen conditions. In addition to the ground beef, a beef system was made containing both histidine and propyl gallate to act as antioxidants.

The data for Run 9 are presented in Tables 36, 37, 38, and 39. The following conclusions can be made.

Meat systems. The major deteriorative factor for meat was non-enzymatic browning. As the moisture content was increased the rate became quite extensive, especially at 50% RH.

The addition of antioxidants had no effect on either peroxide value or browning.

Organoleptically, the treated and control meat samples were best at 0 and 31% RH and were very poor at 50% RH.

Packaging in the different atmospheres did not seem to have much effect on the results, although changes may have occurred if storage time had been longer.

Potato. Although a rancid hay-like odor developed after 3 weeks, the peroxide value determinations were inconclusive.

Browning deterioration seemed to predominate, probably

because the lipid content was very low (0.8% on a dry weight basis). At 50% RH the potatoes were unacceptably dark. Only at 50% RH did packaging in vacuum have any significant effect.

Organoleptically, the potatoes at 0% RH were acceptable while at higher humidities they were not.

Applesauce. Packaging in vacuum caused excessive compression, making it difficult to rehydrate the sample.

Browning was more excessive in the air packs than under vacuum conditions. Production of carbon dioxide and of browning pigments increased with moisture content.

Organoleptically, the only acceptable applesauce was that stored under dry conditions. There was no difference between vacuum and air packaging.

GENERAL

CONCLUSIONS

1. The kinetics of the oxidation of protein and lipids in model systems has been shown to be affected by moisture content; a mathematical prediction of the rates can be made. Lipid oxidation decreased as moisture increased whereas protein oxidation increased.

2. Antioxidants such as propyl gallate and butylated hydroxy toluene were quite variable in their action and seemed to have only limited usefulness. Butylated hydroxy toluene seemed to work best under dry conditions; it may have prooxidant effects at higher relative humidities. Propyl gallate worked better at the higher humidities. In the potato starch model system both antioxidants worked poorly while in the high protein system they had some activity.

3. In the presence of trace metal catalysts such as cobalt, the amino acid histidine acted as a significant antioxidant with increasing action at higher moisture contents. This action was greater than that for a chelating agent such as EDTA.

4. Increasing the amount of oxidizable lipid in a system made it much less stable to oxidation and resulted in typical oxidation kinetics. At low oxidizable lipid concentrations, interaction with protein and other constituents decreased the rate of oxidation significantly and changed oxidation kinetics. In starch systems the retardation effect was less so that lipid oxidation proceeded at a much faster rate, showing that dilution of the lipid with starch is less effective than dilution with protein.

5. Manometric techniques seemed to be the best method of studying oxygen absorption. Peroxide value and headspace determinations gave inconclusive results.

6. In rapidly oxidizing systems browning deterioration can be assumed to be negligible.

7. Measurement of browning by carbon dioxide production was not as reliable a technique as was the extraction of the pigments followed by measurement of the optical density.

8. Contrary to expectations, in dry sucrose systems enough water was available to promote hydrolysis of sucrose and yield reducing sugars. These, in turn, can cause considerable browning.

9. Protein, when added to a sugar-acid system, was significant in retarding the rate of browning, possibly through binding of the water.

10. In general, humidification to slightly below or above the monolayer gave maximum protection to the model system. At 0% RH lipid oxidation was excessive and at 30 to 40% RH browning took place rapidly.

11. The tests of the actual food systems showed that the influence of moisture content was significant whereas packaging environment had only a negligible effect. These results must be considered applicable only to the conditions actually tested (*i.e.*, short-term storage at high temperatures [130°F]). It is most likely that at lower storage temperatures browning would not be as rapid and packaging protection would be quite effective.

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TABLES

1 - 39

TABLE 1

High Fat-High Protein Model System

Component	Percent Composition		Component Used
	Wet Basis	Dry Basis	
Protein	34.5	58.8	Spray-Dried Egg Albumin*
Fat	24.2	41.2	Methyl Linoleate* Methyl Oleate* Apiezon B†
Myoglobin	<0.002	0.002	Horse Myoglobin*
Water	41.3		

* Mann Research Labs, New York.

† J. G. Biddle, Philadelphia, Pennsylvania.

TABLE 2

Low Fat-High Sugar Model System

Component	Percent Composition		Component Used
	Wet Basis	Dry Basis	
Carbohydrates			
Glucose-Sucrose	66.8	86.9	*
Malic Acid	2.0	2.6	*
Fiber-Avicel	4.8	6.2	†
Fat	1.9	2.5	‡
Protein	1.4	1.8	Egg Albumin§
Water	23.1		Redistilled, Deionized

* Mallinckrodt Chemical Company, St. Louis, Missouri.

† American Viscose Corporation, Maryland.

‡ Same as for the high fat-high protein model system.

§ Mann Research Labs, New York.

TABLE 3A

*Low Fat-High Sugar Model System Composition for Run 3**

Component	100% Sucrose	50:50 Glucose-Sucrose
Carbohydrates		
Sucrose	133.6	66.8
Glucose		66.8
Malic Acid	4.0	4.0
Avicel	9.6	9.6
Methyl Oleate	3.8	3.8
Egg Albumin	2.8	2.8
Redistilled Water	46.0	56.0

* Values are in grams.

TABLE 3B

*Moisture Contents (GLC Method)**

System	Dry	31% RH	50% RH
100% Sucrose	0.31	0.50	0.98
50:50 Glucose-Sucrose	5.42	7.39	10.24

* Data are given as g water per 100 g solid.

TABLE 4A

*Composition of the Sucrose Model Systems Used in Run 8**

Component	System I	System II	System III
Sucrose	150	150	150
Avicel	10	10	10
Citric Acid		5	5
Egg Albumin			5
Redistilled Water	40	40	55

* Values are in grams.

TABLE 4B

*Moisture Contents (GLC Method)**

System	Dry	31% RH	75% RH
I	0.11	0.33	0.95
II	0.22	0.47	3.72
III	0.39	3.85	5.67

* Data are given as g water per 100 g solid.

TABLE 5

Composition of the Potato Starch Model Systems Used in Run 6, †*

Component	System: Control (Con)	Propyl Gallate (PG)	Butylated Hydroxy Toluene (BHT)
Potato Starch	45.5	45.5	45.5
Methyl Linoleate	10	10	10
Egg Albumin	4.5	4.5	4.5
Avicel	40	40	40
Cobalt	10^{-5}	10^{-5}	10^{-5}
Propyl Gallate		0.72×10^{-2}	
Butylated Hydroxy Toluene			0.75×10^{-2}
Redistilled Water	200	200	200

* Values are in grams.

† Moisture content by GLC (as g water per 100 g solid): dry, 0.28; 11% RH, 2.66; 31% RH, 5.63.

TABLE 6

Composition of the Potato Starch Model Systems Used in Run 7, †*

Component	System			
	Control (Con)	Cobalt (Co)	Propyl Gallate (PG)	Butylated Hydroxy Toluene (BHT)
Potato Starch	31.8	31.8	31.8	31.8
Lipid				
Methyl Linoleate (30%)	2.1	2.1	2.1	2.1
Apiezon B (70%)	4.9	4.9	4.9	4.9
Egg Albumin	3.2	3.2	3.2	3.2
Avicel	28	28	28	28
Cobalt		1×10^{-5}		
Propyl Gallate			1.51×10^{-3}	
Butylated Hydroxy Toluene				1.57×10^{-3}
Redistilled Water	140	140	140	140

* Values are in grams.

† Moisture content by GLC (as g water per 100 g solid): dry, 0.54; 11% RH, 3.21; 31% RH, 6.04.

TABLE 7

Constant Humidity Solutions

Saturated Salt Solution	Percent Relative Humidity at 55°C
Drierite	<0.1
$LiCl_2$	11.0
CH_3COOK	23.0
$MgCl_2$	31.0
CrO_3	40.2
$Na_2Cr_2O_7$	50.0
NaBr	57.0
$NaNO_3$	61.8
NaCl	75.0
K_2CrO_4	82.0
KNO_3	89.0
$NH_4H_2PO_4$	91.0

TABLE 8

B.E.T.-Data Isotherms

System	M_1*	Percent Relative Humidity at Monolayer	C†
High Fat-High Protein	3.89	31.0	4.12
Low Fat-High Sugar	0.42	25.0	2.00

* M_1 is the monolayer coverage (as g water per 100 g protein).

† C is the B.E.T. activation energy constant.

TABLE 9

High Fat-High Protein Model System
Oxidation Results for Run 1

	Dry	11% RH	31% RH
Initial Extent of Oxidation by UV (µl Oxygen per Gram)	99.5	107.1	84.2
Peroxide Value meq per Kg of Lipid			
Initial	19.4	15.4	14.5
24 hr	235.	157.	90.
54 hr	780.	790.	640.
µl Oxygen per Gram of Lipid			
Initial	217.	172.	162.
24 hr	2630.	1758.	1009.
54 hr	8736.	8848.	7168.
Head-Space Samples (Percent Oxygen)			
Initial	19.8	19.8	19.8
26 hr	17.8	18.4	19.8
54 hr	5.7	5.7	6.8

TABLE 10

High Fat-High Protein Model System
Non-enzymatic Browning Results for Run 1

	Dry	11% RH	31% RH
Pigment Coloration (OD_{420} per Gram x 100)			
Initial	3.7	2.6	1.5
72 hr: Sample 1	1.4	0.9	3.1
72 hr: Sample 2	0.8	2.2	3.2
Head-Space Carbon Dioxide (Percent)			
Initial	0.04	0.04	0.04
24 hr	0.10	0.17	0.35
54 hr	0.50	0.80	1.10

TABLE 11A

*Composition of the High Fat-High Protein Model Systems Used in Run 2**

Component	System: Control (Con)	Propyl Gallate (PG)	Butylated Hydroxy Toluene (BHT)
Egg Albumin	58.8	58.8	58.8
Lipid			
Methyl Oleate (50%)	20.5	20.5	20.5
Methyl Linoleate (10%)	4.2	4.2	4.2
Apiezon B (40%)	16.5	16.5	16.5
Myoglobin	2×10^{-5}	2×10^{-5}	2×10^{-5}
Propyl Gallate		5×10^{-3}	
Butylated Hydroxy Toluene			5×10^{-3}
Redistilled Water	70	70	70

* Values are in grams.

TABLE 11B

*Moisture Contents**

	Dry	31% RH	50% RH
Moisture Content by Vacuum	2.38	5.81	7.53
Moisture Content by GLC	1.43	4.68	5.53

* Data are given as g water per 100 g solid.

TABLE 12

Oxygen Absorption by the Lipid in Run 2, †*

Time (hr)	Control			PG			BHT		
	Dry	31%	50%	Dry	31%	50%	Dry	31%	50%
20	120	0	100	100	100	0	200	300	100
50	250	50	375	350	250	0	500	700	250
80	600	100	600	550	300	0	700	950	300
100	800	200	1000	650	350	0	725	1050	400
150	1100	600	1650	700	475	0	800	1175	475
200	1400	950	2050	800	550	0	850	1200	600
250	1600	1150	2250	875	600	0	875	1300	750
300	1750	1325	2425	925	700	0	925	1350	850
350	1900	1475	2550	1000	750	0	1050	1450	
400	2050	1650	2675	1050	850	0		1500	

* Average of duplicate samples.

† Data are given as microliters of oxygen per gram of linoleate.

TABLE 13

*Peroxide Values for Run 2**

Time (hr)	Dry			31% RH			50% RH		
	Con	PG	BHT	Con	PG	BHT	Con	PG	BHT
0	6	45.4	47.6	0	47.6	67.5	30.8	52.6	61.5
168	15.6	50.0	29.8	21.0	32.4	50.0	59.9	57.6	52.2
384	34.9	50.0	46.7	30.1	36.7	43.7	39.4	52.6	40.2
504	21.9	59.6	74.0	21.6	41.9	30.4	24.5	53.1	23.8

* Values are given as milliequivalents of oxygen per kilogram of linoleate.

TABLE 14A

*Carbon Dioxide Production in Run 2**

Time (hr)	Dry			31% RH			50% RH		
	Con	PG	BHT	Con	PG	BHT	Con	PG	BHT
0	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.130	0.090
11.5	0.049	0.045	0.049	0.04	0.056	0.067	0.054	0.135	0.112
103.5	0.054	0.045	0.049	0.075	0.056	0.040	0.094	0.049	0.108
177.5	0.056	0.056	0.045	0.067	0.078	0.062	0.090	0.067	0.056
295.5	0.056	0.056	0.045	0.067	0.067	0.058	0.081	0.045	0.049

* Percent concentration in head space.

TABLE 14B

*Browning Data for Run 2**

Time (days)	Dry			31% RH			50% RH		
	Con	PG	BHT	Con	PG	BHT	Con	PG	BHT
0	2.61	5.95	2.0	1.96	1.95	4.98	2.10		2.53
15	4.31	0.49	4.51	4.95	2.28	0	2.06	2.0	0

* (OD_{420} mμ per gram)(*x* 100).

TABLE 15

Composition of the High Fat-High Protein Model Systems Used in Run 4, †*

Component	System: Control (Con)	Propyl Gallate (PG)	Butylated Hydroxy Toluene (BHT)
Egg Albumin	58.8	58.8	58.8
Lipid			
Methyl Oleate (30%)	12.35	12.35	12.35
Methyl Linoleate (30%)	12.35	12.35	12.35
Apiezon B (40%)	16.5	16.5	16.5
Cobalt‡	0.6 mg	0.6 mg	0.6 mg
Propyl Gallate§		15 mg	
Butylated Hydroxy Toluene§			14.75 mg
Redistilled Water	80	80	80

* Values are in grams.

† Moisture content by GLC (as g water per 100 g solid; average of three samples): 0.1% RH, 0.495; 31% RH, 3.88; 60% RH, 6.90.

‡ As a nitrate salt solution. 0.610 g made to 100 ml. 1 ml ⟶ 10 ppm cobalt based on linoleate.

§ 0.3 g made to 100 ml with water. 5 ml ⟶ 10^{-3} moles propyl gallate per mole of linoleate.

§ 29.5 mg made to 10 ml with Apiezon B. 5 ml ⟶ 10^{-3} moles butylated hydroxy toluene per mole of linoleate.

TABLE 16

Oxygen Absorption by the Lipid in Run 4, †*

Time (hr)	Control			PG			BHT		
	Dry	31%	60%	Dry	31%	60%	Dry	31%	60%
24.5	284	240	230	440	126	252	290	111	341
49	411	467	372	590	295	616	408	290	697
102	986	952	730	1000	609	1289	762	584	1353
140	1481	1240	1109	1281	807	1746	960	748	1714
189	2234	1548	1602	2134	950	1950	1558	792	2107
241	3648	2070	2458	3265	1200	2000	2545	900	2700
297	6757	3161	3524	5084	1572	2296	4944	1182	3269
337	11347	3896	4555	7549	1799	2400	9615	1309	3943
359	13508	4528	5109	9653	1973	2647	12098	1441	4253
408	16339	5063	5697	11397	2144	3024	15271	1555	4609
428		6449	7238		2558	3085		1892	5374

* Average of duplicate samples.

† Data are given as microliters of oxygen per gram of linoleate.

TABLE 17

Head-Space Oxygen Concentration (%) in Run 4

Time (Days)	Control			PG			BHT		
	Dry	31%	60%	Dry	31%	60%	Dry	31%	60%
0	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8	19.8
2	19.8	19.8	19.8	19.4	19.5	19.4	19.8	18.7	19.8
5	18.5	19.2	18.7	18.4	19.2	18.0	18.7	19.0	18.6
7	19.4	19.5	19.4	19.8	19.4	18.9	19.4	19.6	19.3
8	19.0	19.4	18.9	19.0	19.2	18.4	19.1	19.1	19.3
12	18.4	18.7	18.4	18.4	18.4		18.5	18.7	
14	18.9	18.6	18.5	19.4	19.4	18.8	19.8	19.9	19.4
20	17.6	17.6	17.1	17.6	19.5	18.7	17.1	17.3	18.3

TABLE 18

*Peroxide Values for Run 4**

Time (Days)	Control			PG			BHT		
	Dry	31%	60%	Dry	31%	60%	Dry	31%	60%
0	10.5	6.4	11.5	11.6	10.7	13.3	17.1	15.2	14.6
3	5.1	11.8	10.7	9.2	14.0	7.0	3.6	13.2	10.7
8	12.9	23.6	9.8	16.8	13.5	6.8	7.1	9.3	6.2
14	26.5	15.6	5.1	26.1	11.1	7.7	19.1	4.0	5.6
16	29.4	22.7	18.1	37.4	11.5	2.4	23.6	8.1	15.4
20	58.0		25.8	15.0	19.3	7.4	25.8	6.4	10.2
22	27.5	50.1	61.4	91.8	12.5	10.6	9.4	9.2	16.2

* Values are given as milliequivalents of oxygen per kilogram of linoleate.

TABLE 19

*Development of Browning in Run 4**

Time (Days)	Control			PG			BHT		
	Dry	31%	60%	Dry	31%	60%	Dry	31%	60%
0	1.07	1.11	1.19	0.85	1.54	1.17	1.14	1.00	0.95
7	1.29	2.89	1.81	1.16	1.11	1.12	1.21	2.43	3.58
15	1.01	0.95	1.25	1.36	1.00	0.71	0.85	0.96	0.82
20	1.87	1.00	1.67	1.52	5.50	1.26	4.61	1.10	2.32

* (OD per gram) x 100.

TABLE 20

*Carbon Dioxide Production in Run 4**

Time (Days)	Control			PG			BHT		
	Dry	31%	60%	Dry	31%	60%	Dry	31%	60%
0	0.04	0.04	0.18	0.04	4.4	4.32	0.04	0.06	0.06
2	0.04	0.14	0.07	3.38	2.8	3.31	0.06	0.06	0.05
5	0.06	0.08	0.04	2.30		2.97	0.09	0.08	0.07
7	0.06	0.08	0.07		1.28	4.77	0.14	0.08	0.09
8	0.09	0.08	0.09	1.51	1.06	4.28	0.06	0.09	0.08
12	0.10	0.09	0.09	1.17	0.52	3.54	0.11	0.07	0.09
14	0.10	0.10	0.18	0.68	0.31	1.00	0.05	0.09	0.08
20	0.10	0.06	0.25	1.22		0.29	0.13	0.09	0.14

* Percent concentration in head space.

TABLE 21

Composition of the High Fat-High Protein Model Systems Used in Run 5, †*

Component	System					
	Control	Cobalt	Histidine	EDTA	Cobalt + EDTA	Cobalt + Histidine
Egg Albumin	29.4	29.4	29.4	29.4	29.4	29.4
Lipid						
Methyl Oleate (20%)	4.12	4.12	4.12	4.12	4.12	4.12
Methyl Linoleate (40%)	8.24	8.24	8.24	8.24	8.24	8.24
Apiezon B (40%)	8.24	8.24	8.24	8.24	8.24	8.24
Cobalt‡		0.407 mg			0.407 mg	0.407 mg
Histidine§			4.3 mg			4.3 mg
EDTA¶				5.2 mg	5.2 mg	
Water	40 ml	40 ml	40 ml	40 ml	40 ml	40 ml

FOOTNOTES TO TABLE 21

* Values are in grams.

† Moisture content by GLC (as g water per 100 g solid; average of six samples): <0.1% RH, 0.135; 31% RH, 3.94; 40% RH, 4.54.

‡ 0.407 g cobalt nitrate made to 1000 ml. 1 ml ⟶ 10 µg cobalt per gram of linoleate.

§ 0.0434 g histidine made to 100 ml. 10 ml ⟶ 10^{-3} moles per mole of linoleate.

§ 0.5205 g EDTA made to 100 ml. 1 ml ⟶ 10 moles per mole of cobalt.

TABLE 22

*Oxygen Absorption by the Lipid in Run 5**

Time (hr)	Control		Cobalt		Histidine		EDTA		Cobalt + EDTA		Cobalt + Histidine	
	31%	40%	31%	40%	31%	40%	31%	40%	31%	40%	31%	40%
24.5	67	197	76	150	19	154	79	118	96	68	34	35
66.0	301	592	266	950	226	456	264	577	466	372	175	168
97.5	669	1068	667	2296	489	751	496	750	961	947	396	315
121.5	1317	1458	1262	3664	835	1105	704	961	1511	1721	629	472
150.5	2510	2426	2253	6134	1692	1853	890	1395	2222	3007	830	914
186.5	4564	3406	4010	9586	2043	3227	1075	2407	3275	5189	1031	1632
201.5	5560	3884	4999	11471	2255	3853	1271	3386	3810	6304	1196	2072
217.5	6038	4436	5831	13453	2760	4758	1523	4363	4723	7525	1433	2565
235	7265	5407	7866	15229	3053	5816	1660	5252	5495	8500	1677	3155

* Data are given as microliters of oxygen per gram of linoleate.

TABLE 23A

*Peroxide Values for Run 5**

Time (Days)	Control			Cobalt			Histidine		
	Dry	31%	40%	Dry	31%	40%	Dry	31%	40%
3	8.51			30.6			19.4		
4			12.0			11.7			13.1
7		20.5			40.0			20.5	
9	53.0			56.6			41.4		
10			19.0			27.8			18.9
11		30.0			62.0			21.5	

* Values are given as milliequivalents of oxygen per kilogram of linoleate.

TABLE 23B

*Peroxide Values for Run 5**

Time (Days)	EDTA			Cobalt + EDTA			Cobalt + Histidine		
	Dry	31%	40%	Dry	31%	40%	Dry	31%	40%
3	15.4			15.2			14.0		
4						16.0			9.8
7		12.6			27.5			19.1	
9	73.1			67.7			60.8		
10		31.7				21.8			15.5
11					34.6			20.4	

* Values are given as milliequivalents of oxygen per kilogram of linoleate.

TABLE 24

Estimated Percent Sucrose Hydrolysis

Sample	Tests	
	Reducing Sugar	Glucose Oxidase
Frozen Control	1.9	1.2
Stored at 55°C for 21 Days		
Dry	4.0	3.1
31% RH	6.1	5.7
50% RH	13.3	7.5

TABLE 25

*Carbon Dioxide Production in Run 3**

Time (Days)	100% Sucrose			50:50 Glucose-Sucrose		
	Dry	31%	51%	Dry	31%	51%
0	0.067	0.084	0.067	0.045	0.056	0.073
1	0.078	0.101	0.090	0.040	0.073	0.090
3	0.090	0.112	0.112	0.067	0.067	0.123
6	0.101	0.135	0.180	0.067	0.084	0.112
7	0.072	0.112	0.146	0.056	0.078	0.067
8	0.112	0.123	0.130	0.073	0.108	0.090
10	0.067	0.103	0.099	0.045	0.157	0.099
13	0.090	0.067	0.281	0.135	0.180	0.168
14	0.092	0.067	0.247	0.180	0.247	0.135
16	0.067	0.203	0.293	0.079	0.146	0.169
17	0.158	0.067	0.079	0.135	0.239	0.180
24	0.225	0.067	0.135	0.079	0.077	0.111
31	0.067	0.045	0.101	0.090	0.338	0.135

* Percent concentration in head space.

TABLE 26

Estimated Percent Sucrose Hydrolysis in Run 8

Time (Days)	System I			System II			System III		
	Dry	31%	75%	Dry	31%	75%	Dry	31%	75%
0	0.15	0.05	0.06	1.45	1.81	8.05	0.91	0.96	2.43
3	0.09	0.03	0.09	2.83	6.62	41.3	1.13	4.79	11.96
7	0.13	0.06	0.15	5.26	8.14	70.4	0.85	15.87	21.68
10	0.12	0.05	0.11	5.22	6.51	69.6	2.91	14.42	16.30
14	0.13	0.07	0.12	7.89	10.97	73.8	2.81	15.58	25.80
17							2.81	18.06	40.37
22							3.25	16.54	36.05

TABLE 27

*Carbon Dioxide Production in Run 8**

Time (Days)	System I			System II			System III		
	Dry	31%	75%	Dry	31%	75%	Dry	31%	75%
3	0.045	0.062	0.067	0.058	0.063	0.103	0.054	0.076	0.076
7	0.058	0.063	0.063	0.072	0.058	0.076	0.051	0.069	0.072
10	0.047	0.054	0.047	0.060	0.045	0.108	0.036	0.076	0.081
14	0.054	0.052	0.063	0.054	0.083	0.063	0.058	0.072	0.065
17	0.054	0.063	0.054	0.094	0.110	0.135	0.063	0.058	0.121

* Percent concentration in head space.

TABLE 28

*Oxygen Absorption by the Lipid in Run 6**

Time	Dry			11% RH			31% RH		
(hr)	Con	PG	BHT	Con	PG	BHT	Con	PG	BHT
7.5	260	92	203	20	40	80	102	47	110
13.25	560	288	485	39	119	201	249	177	242
19.0	860	623	795	234	280	489	389	272	353
24.5	1261	1190	1056	443	418	576	625	449	507
32	2680	2645	1429	639	667	871	840	698	684
35.5	5278	4620	1951	1043	855	1032	994	911	794
39.0	8530	7522	3020	1591	1005	1220	1178	1247	871
41.0	1308	10006	4462	2217	1194	1421	1322	1467	960
43.0	20200	13745	6849	2973	1432	1689	1445	1751	1070
45.0				4017	1681	1877	1588	2011	1191
47.0				5504	2049	2198	1721	2272	1345
49.5				8569	2576	2016	1937	2650	1566
50.5					3074	3406	2049	2887	1710
52.5					3850	4934	2428	3021	2118
54.0					4845	6717	2695	3786	2416
57.0					7302	11142	3689	4733	3431

* Data are given as microliters of oxygen per gram of linoleate.

TABLE 29

*Peroxide Values for Run 6**

Time (hr)	Dry			11% RH			31% RH		
	Con	PG	BHT	Con	PG	BHT	Con	PG	BHT
0	6.7	3.0	3.3	3.8	1.6	3.0	3.5	2.9	2.5
48	30.2	35.	15.6	20.	14.	22.	35.	20.	25.
72	1929.	86.	926.	64.	40.	141.	161.	44.	157.
96	1969.	719.	1467.	952.	104.	1112.	330.		886.
108	1455.	1048.	822.	1607.	558.	1138.	1853.	316.	988.

* Values are given as milliequivalents of oxygen per kilogram of linoleate.

TABLE 30A

*Oxygen Absorption at <0.1% RH in Run 7**

Time (hr)	Control		Cobalt		Propyl Gallate		Butylated Hydroxy Toluene	
	#150	#27	#222	#17	#74	#60	#4	#1
7	96	87	1870		84	198	114	141
12	450	174	2684	295	421	395	432	406
25	900	1090	5278	1542	1138	1186	1092	1696
30.5	1254	1701	6243	2270	1686	1779	1751	2331
37	1511	2050	6846	3223	1854	1977	2138	3127
41	1543	2094	7208	3881	1981	2254	2411	3621
49	1608	2181	7570	5042			3002	4734
64.5	2154	2705	8896	7814	2402	3044		7136
71	2347	2835	9289	8905	2529		4048	8320

* Data are given as microliters of oxygen per gram of linoleate.

TABLE 30B

*Oxygen Absorption at <0.1% RH in Run 7**

Time (hr)	Control		Cobalt		Propyl Gallate		Butylated Hydroxy Toluene	
	#150	#27	#222	#17	#74	#60	#4	#1
88.5	3022	3359	10284	11556		3176	5822	10051
95	3344	3577	10676	12492	2992	4033	6527	11341
103.5	3922	3926	11490	14952	3245	4230	8414	14397
112	4533	4580	14265	16321	3583	4744	10234	16728
127.5	5240	4842	15199	19145		5219	12508	21127
136.5	5691	5191	15531	21206	3878	5614	14100	24183
143.5	6141	5453	15742	22488	3962	5931	15396	26638
149	6559	5976	16255	23684	4383	6484	16465	28811
162	7009	6238	16587	26768		6919	19194	33227

* Data are given as microliters of oxygen per gram of linoleate.

TABLE 31A

*Oxygen Absorption at 11% RH in Run 7**

Time (hr)	Control		Cobalt		Propyl Gallate		Butylated Hydroxy Toluene	
	#503	#20	#500	#322	#502	#278	#7	#5
7								
12	250	107	247	483	190		194	435
25	437	320	525	886	412	183	401	991
30.5		640	680	1047	601		470	1402
37	874	712	834	1127	791	330	609	1910
41	998		896	1208	823	366	705	2248
49	1279		958	1248	855	440	1024	3319
64.5	2184	1139	1545	2094	1266	952	2227	4956
71	2465	1281	1669	2255	1393	1172	2739	5633

* Data are given as microliters of oxygen per gram of linoleate.

TABLE 31B

*Oxygen Absorption at 11% RH in Run 7**

Time (hr)	Control		Cobalt		Propyl Gallate		Butylated Hydroxy Toluene	
	#503	#20	#500	#322	#502	#278	#7	#5
88.5	3307	1779	2287	2940	1868	1868		6116
95	3588	2064	2565	3141	2058	2088	3223	6406
103.5	4275	2348	2967	3584	2406	2930		7929
112	4774	2811	3492	4067	3007	3663		8582
127.5	5304	3060	3925	4671	3129	4102	4228	10467
136.5	5773	3380	4357	4953	3387	4285	5395	11676
143.5	6334	3701	4790	5356	3704	4688	6128	12643
149	6740	4092	5130	5678	3926	5018	6640	
162	7520	4305	5624	5960	4020	5274	7954	13634

* Data are given as microliters of oxygen per gram of linoleate.

TABLE 32A

*Oxygen Absorption at 31% RH in Run 7**

Time (hr)	Control		Cobalt		Propyl Gallate		Butylated Hydroxy Toluene	
	#176	#10	#217	#271	#501	#253	#17	#8
7							720	
25	110	25	35	39	268	261	3499	452
30.5		75	173		536		3877	957
37	442	175	451	232	803	745	4426	1462
41	479		485	348	918	857	4460	1835
49	589		624		1109	1044	4837	2500
64.5	1252	626	1387	968	1989	2087	8268	4495
71	1694	877	1734	1471	2410	2572	10327	5426

* Data are given as microliters of oxygen per gram of linoleate.

TABLE 32B

*Oxygen Absorption at 31% RH in Run 7**

Time (hr)	Control		Cobalt		Propyl Gallate		Butylated Hydroxy Toluene	
	#176	#10	#217	#271	#501	#253	#17	#8
88.5	2394	1478	2635	2284	3405	3541	15816	7660
95	2678	1654	3051	2478	3868	3988	16056	8404
103.5	2836	2254	3814	2865	4706	4584	19727	10213
112	3168	2681	4334	3213	5203	5031	22266	11516
127.5	4383	4159	4756	4413	7001	6709	27891	13777
136.5	4862	4610	6380	4878	7843	7342	31974	15292
143.5	5083	4886	6761	5110	8264	7568	39865	16277
149	5451	5237	7247	5459	8837	8162	45766	
162	6261	5537	8426	6272	10253	9206	52182	17899

* Data are given as microliters of oxygen per gram of linoleate.

TABLE 33

*Peroxide Values for Run 7**

Time (hr)	Control (Con)	Cobalt (Co)	Propyl Gallate (PG)	Butylated Hydroxy Toluene (BHT)
			Dry	
0	4.97	4.54	13.05	9.73
48	5.62	7.78	10.89	4.47
192	31.52	18.45	68.45	23.52
240	34.09	35.17	31.53	115.62
			11% RH	
0	5.83	11.77	5.93	11.27
48	4.83	14.90	4.60	9.76
192	21.84	12.96	7.67	10.98
240	35.07	20.60	24.43	25.46
			31% RH	
0	5.25	4.12	8.70	15.01
48	6.16	9.62	4.67	4.17
192	10.49	17.90	16.90	27.70
240	19.41	18.22	14.12	15.23

* Values are given as milliequivalents of oxygen per kilogram of linoleate.

TABLE 34

*Monomolecular Rate Constants**

	Dry	11% RH	31% RH
		Run 6	
Control	2.62	1.94	1.84
Propyl Gallate	2.76	1.62	1.80
Butylated Hydroxy Toluene	3.04	1.88	1.44
		Run 7	
Control	1.54	1.32	1.00
Cobalt	3.14	0.94	1.48
Propyl Gallate	2.72	1.10	1.76
Butylated Hydroxy Toluene	2.76	0.94	2.86

* (Microliters of oxygen per gram)$^{\frac{1}{2}}$ hour^{-1}.

TABLE 35A

*Moisture Contents of Freeze-Dried Foods**

	Dry	31% RH	50% RH
Meat	0.72	4.14	6.00
Meat + Propyl Gallate + Histidine	0.70	4.27	5.40
Potato	0.67	3.54	4.83
Applesauce	2.64	5.50	8.37

* Data are given as g water per 100 g solid.

TABLE 35B

Monolayer Values

	m_o*	Percent Relative Humidity
Meat	2.85	20
Meat + Propyl Gallate + Histidine	2.85	20
Potato	3.5	32
Applesauce	4.6	22

* As grams of water per 100% RH.

TABLE 36

*Peroxide Values for Run 9**

Time	Dry			31% RH			50% RH		
	Vac	Mid	Air	Vac	Mid	Air	Vac	Mid	Air
	Meat								
Initial	0			1.11			1.11		
10 Days	0.55	0.55	1.11	0.55	0.55	1.11	1.11	1.11	1.11
3 Weeks	0.55	1.67	1.11	1.11	1.11	1.11	0.55	1.11	1.11
	Meat + Propyl Gallate + Histidine								
Initial	1.11			0.55			0.55		
10 Days	1.11	1.11	1.67	1.67	2.78	1.11	0.55	2.78	2.78
3 Weeks	0.55	1.67	2.22	0.55	1.11	2.22	0.55	1.11	2.22
	Potato								
Initial	13			13			13		
10 Days	0	0	0	13	0	13	13	0	13
3 Weeks	0	0	13	13	0	13	0	13	13

* Values are given as milliequivalents of oxygen per kilogram of fat.

TABLE 37A

*Browning Data for Run 9**

Time	Dry			31% RH			50% RH		
	Vac	Mid	Air	Vac	Mid	Air	Vac	Mid	Air
	Meat								
Initial	14.4			15.0			13.9		
10 Days	16.25	14.45	12.75	29.0	15.0	16.6	39.95	35.50	32.75
3 Weeks	17.25	13.6	14	36.95	27.5	30.9	55	50	43
	Meat + Propyl Gallate + Histidine								
Initial	14.5			13.6			13.1		
10 Days	14.25	10.55	12.75	21.1	16.9	20.5	31.0	32.0	23.9
3 Weeks	16.1	14.45	13.75	31.0	20.6	25.1	95	36	35.1

* $(OD_{400}$ per gram) x 100.

TABLE 37B

*Browning Data for Run 9**

Time	Dry			31% RH			50% RH		
	Vac	Mid	Air	Vac	Mid	Air	Vac	Mid	Air
	Potato								
Initial	6.9			5.13			4.4		
10 Days	4.5	6.83	4.66	16.0	10.5	13.66	24.66	28.0	19.0
3 Weeks	7.5	8.15	8.0	14.5	19.9	12.1	55.0	81.0	85.0
	Applesauce								
Initial	0.7			0.95			0.98		
10 Days	6.83		9.06	6.66		9.4	7.83		9.7
3 Weeks	9.20		14.9	11.95		18.1	11.4		26.4

* (OD_{400} per gram) x 100.

TABLE 38A

Head-Space Composition in Run 9

	Time	Dry			31% RH			50% RH		
		% O_2	% CO_2	"Hg*	% O_2	% CO_2	"Hg*	% O_2	% CO_2	"Hg*
		Meat								
Air Pack	Initial	20.9	0.04		20.9	0.04		20.9	0.04	
	10 Days	20.8	0.10		21.01	0.13		14.21	3.03	
	3 Weeks	21.3	0.09		21.32	0.06		9.43	5.95	
Middle Pack	Initial	20.3	0.04	12	18.48	0.21	5	19.74	0.08	5
	10 Days	20.6	0.16	10	21.01	0.10	15	1.44	3.88	10
	3 Weeks	21.5	0.16	20	21.11	0.09	25	1.84	4.65	15
		Meat + Propyl Gallate + Histidine								
Air Pack	Initial	20.9	0.04		20.9	0.04		20.9	0.04	
	10 Days	17.64	0.58		15.33	1.86		14.07	2.15	
	3 Weeks	21.73	0.09		15.37	2.58		12.05	3.99	
Middle Pack	Initial	21.21	0.26	8	12.81	0.04	13	17.43	0.04	15
	10 Days	19.53	0.08	15	19.95	0.10	7	0.73	5.08	22
	3 Weeks	18.45	0.82	10	21.11	0.45	7	10.15	3.40	17

* Package vacuum.

TABLE 38B

Head-Space Composition in Run 9

	Time	Dry			31% RH			50% RH		
		% O_2	% CO_2	"Hg*	% O_2	% CO_2	"Hg*	% O_2	% CO_2	"Hg*
		Potato								
Air Pack	Initial	20.9	0.04		20.9	0.04		20.9	0.04	
	10 Days	21.0	0.212		20.79	0.10		19.74	1.09	
	3 Weeks	20.98	0.478		21.38	0.13		20.19	1.51	
Middle Pack	10 Days	20.58	0.159	5	20.58	0.18	7	20.79	0.10	7
	3 Weeks	21.18	0.106	5	21.38	0.09	14	20.79	1.19	8
		Applesauce								
Air Pack	Initial	20.9	0.04		20.9	0.04		20.9	0.04	
	10 Days	21.0	0.24		20.58	0.13		20.16	0.47	
	3 Weeks	20.98	0.45		20.09	0.58		19.0	1.15	

* Package vacuum.

TABLE 39A

Organoleptic Scores for Freeze-Dried Foods in Run 9

	Time	Color			Flavor			Odor		
		Dry	31%	50%	Dry	31%	50%	Dry	31%	50%
		Meat								
Air Pack	Initial	9	9	9	9	9	9	9	9	9
	3 Weeks	7	5	5	7	7	7	6	5	1
Middle Pack	Initial	9	9	9	9	9	9	9	9	9
	3 Weeks*	8	6	6	7	7	7	5	4	3
Vacuum Pack	Initial	9	9	9	9	9	9	9	9	9
	3 Weeks*	8	8	8	7	7	7	6	5	4
		Meat + Propyl Gallate + Histidine								
Air Pack	Initial	9	9	9	9	9	9	9	9	9
	3 Weeks†	8	8	5	7	7	7	3	5	2
Middle Pack	Initial	9	9	9	9	9	9	9	9	9
	3 Weeks†	7	8	5	7	7	7	3	5	2
Vacuum Pack	Initial	9	9	9	9	9	9	9	9	9
	3 Weeks†	8	8	6	7	7	7	7	8	2

Comments: * - rancid odor; † - burnt odor.

TABLE 39B

Organoleptic Scores for Freeze-Dried Foods in Run 9

	Time	Color			Flavor			Odor		
		Dry	31%	50%	Dry	31%	50%	Dry	31%	50%
		Potato								
Air Pack	Initial	9	9	9	9	9	9	8	8	8
	3 Weeks*	9	8	1	5	6	1	6	6	1
Middle Pack	Initial	9	9	9	9	9	9	8	8	8
	3 Weeks	9	8	1	5	4	1	6	6	1
Vacuum Pack	Initial	9	9	9	9	9	9	8	8	8
	3 Weeks	9	7	3	7	6	3	6	4	1
		Applesauce								
Air Pack	Initial	8	8	8	8	8	8	8	8	8
	3 Weeks†	5	3	1	4	2	1	5	5	2
Vacuum Pack‡	Initial	8	8	8	8	8	8	8	8	8
	3 Weeks	5	3	1	3	3	1	5	5	2

Comments: * - hay-like rancid odor; † - burnt sugar taste; ‡ - vacuum-compressed sample, hard to rehydrate.

FIGURES

1 - 33

FIGURE 1

WATER ADSORPTION ISOTHERMS
MODEL SYSTEMS

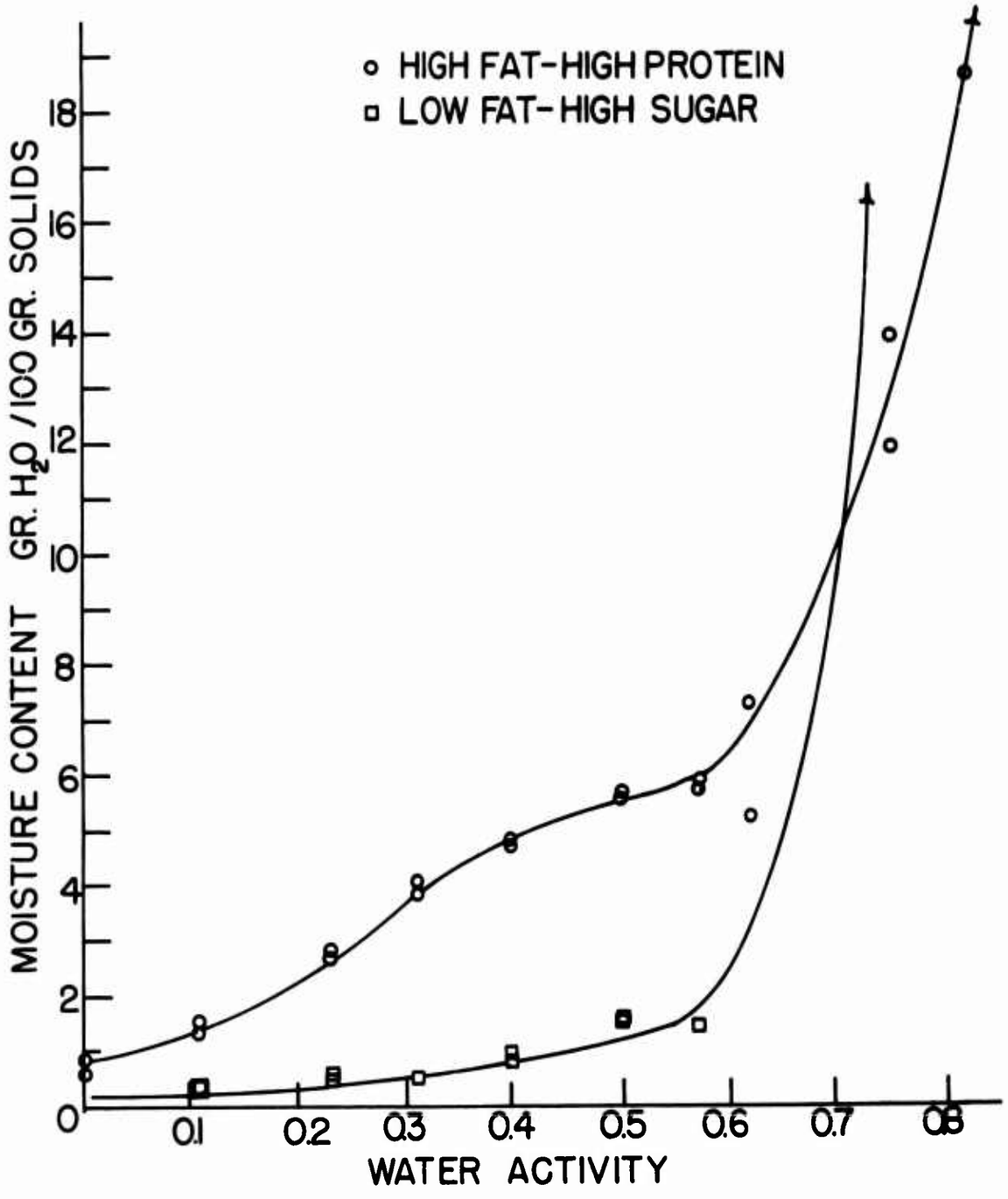


FIGURE 2
RATE OF EQUILIBRATION
HIGH FAT - HIGH PROTEIN SYSTEM

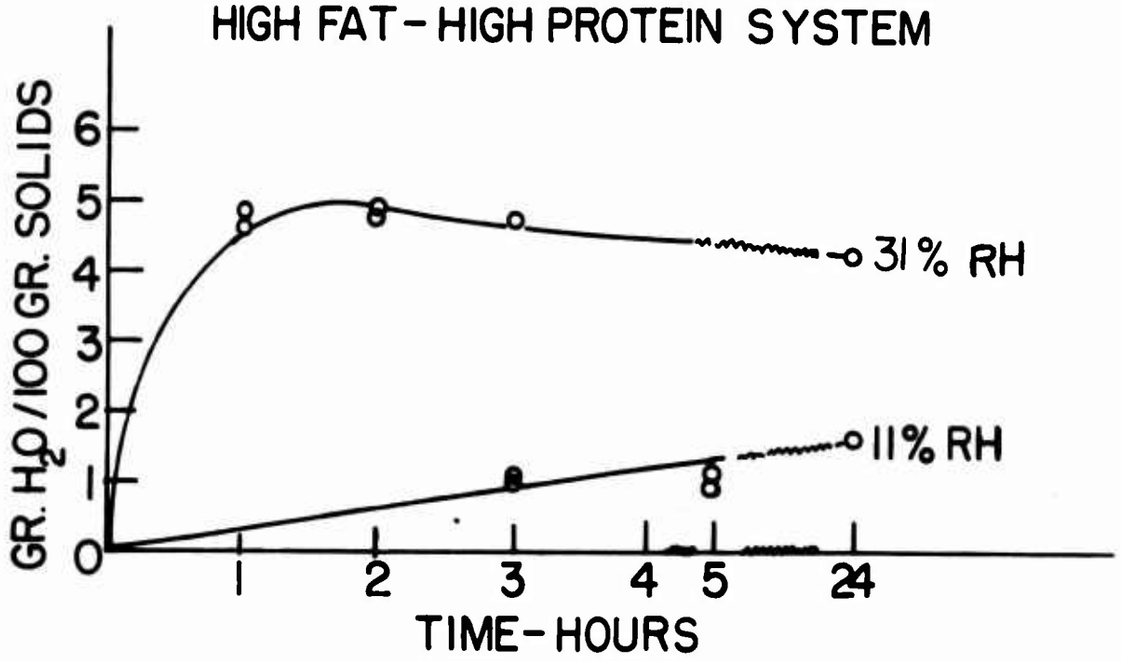


FIGURE 3

POTATO STARCH MODEL SYSTEM

55°C

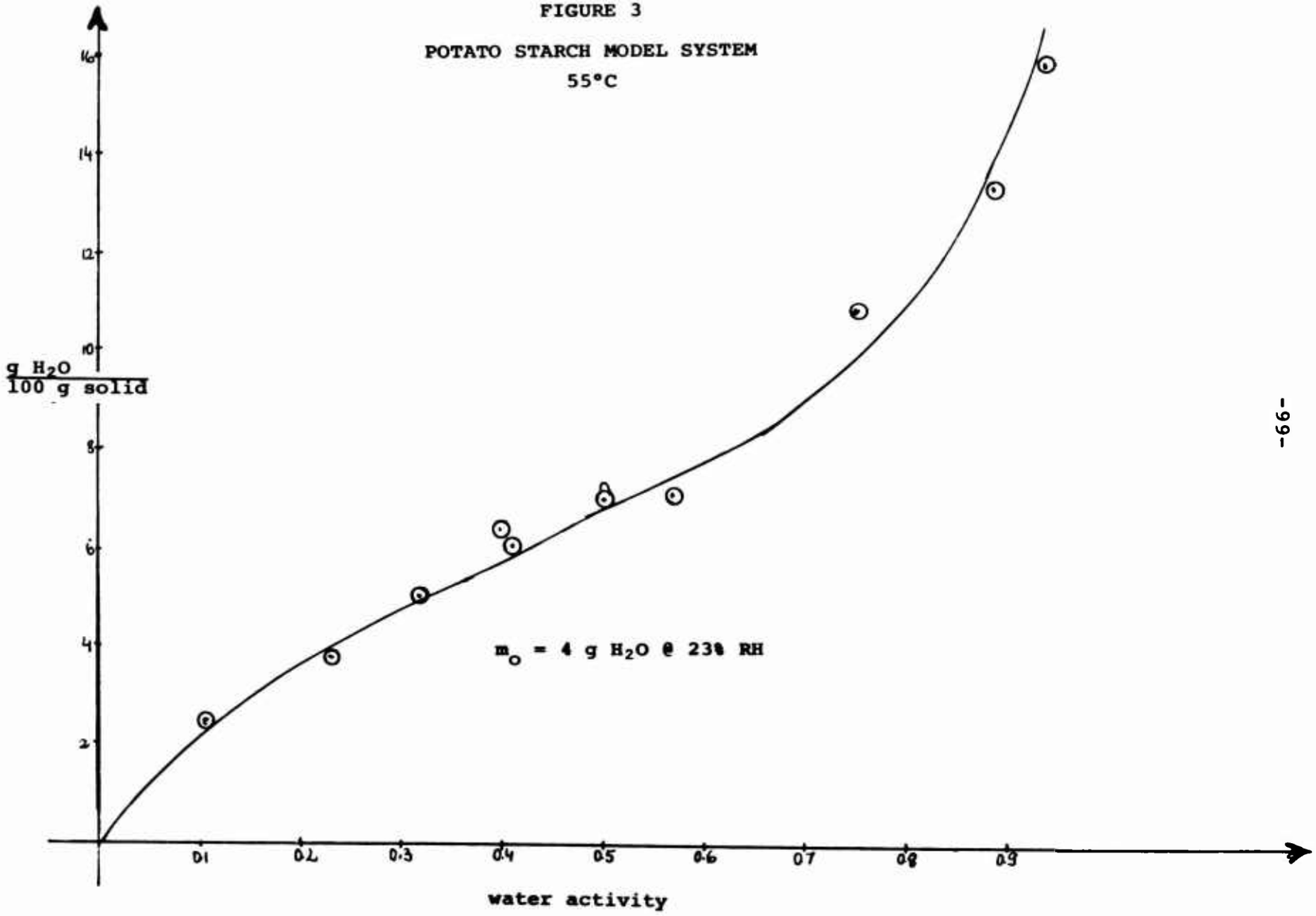


FIGURE 4

OXYGEN ABSORPTION BY PROTEIN

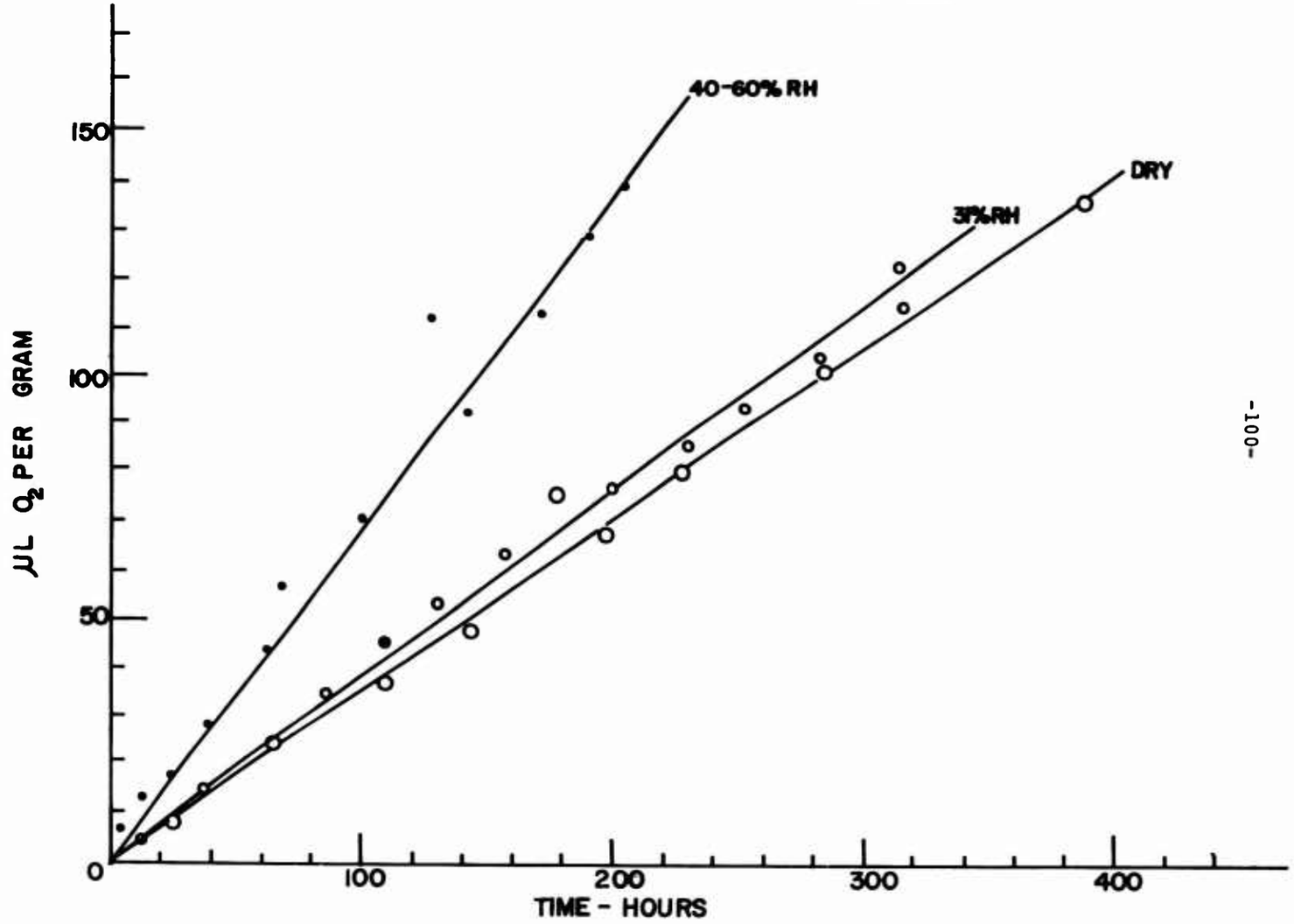


FIGURE 5

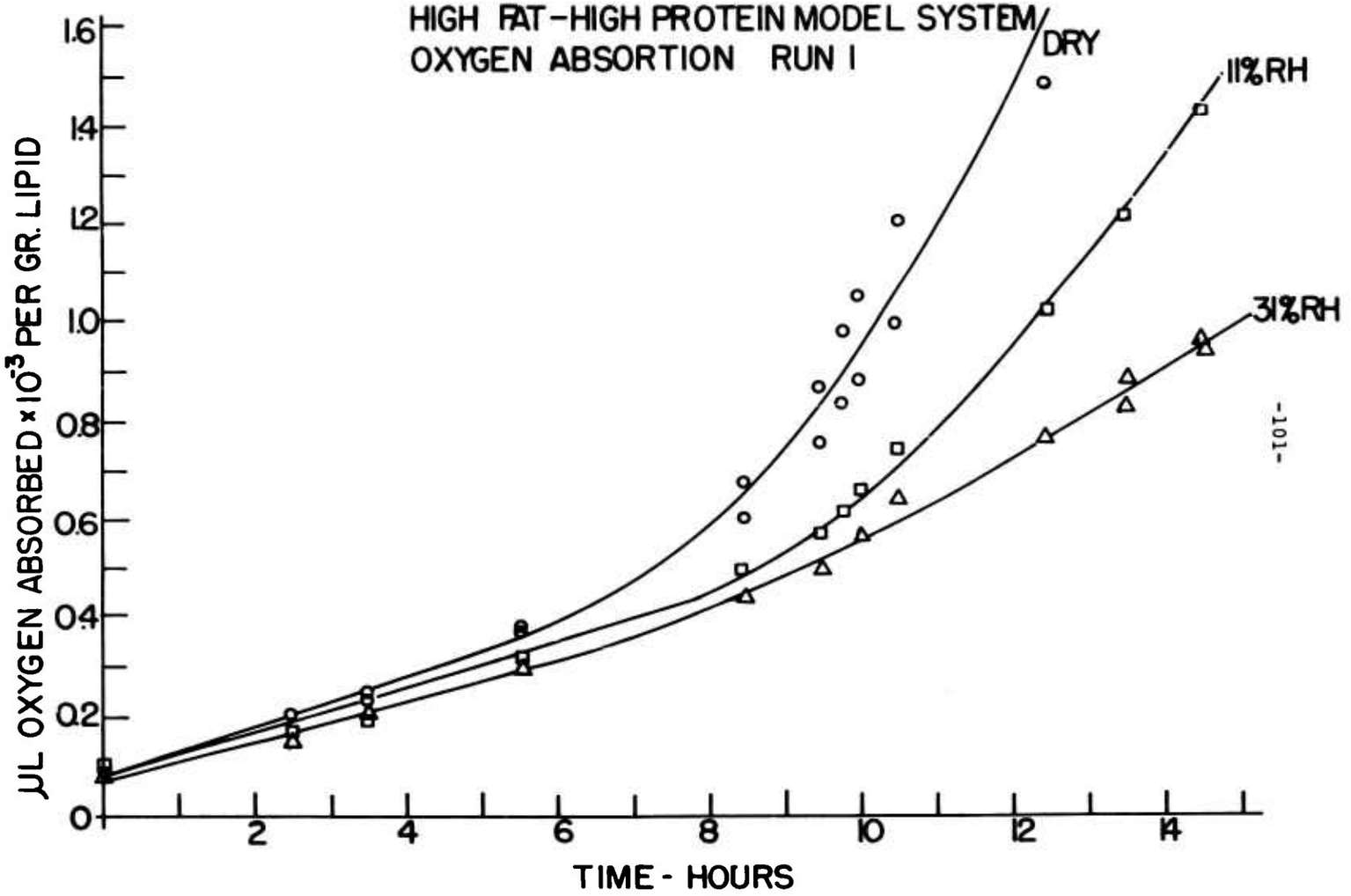

HIGH FAT-HIGH PROTEIN MODEL SYSTEM
OXYGEN ABSORTION RUN I
DRY
11%RH
31%RH
JUL OXYGEN ABSORBED x 10⁻³ PER GR. LIPID
TIME - HOURS
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
2
4
6
8
10
12
14

FIGURE 6
MONOMOLECULAR RATE PLOT
RUN I
HIGH-FAT-HIGH PROTEIN MODEL SYSTEM

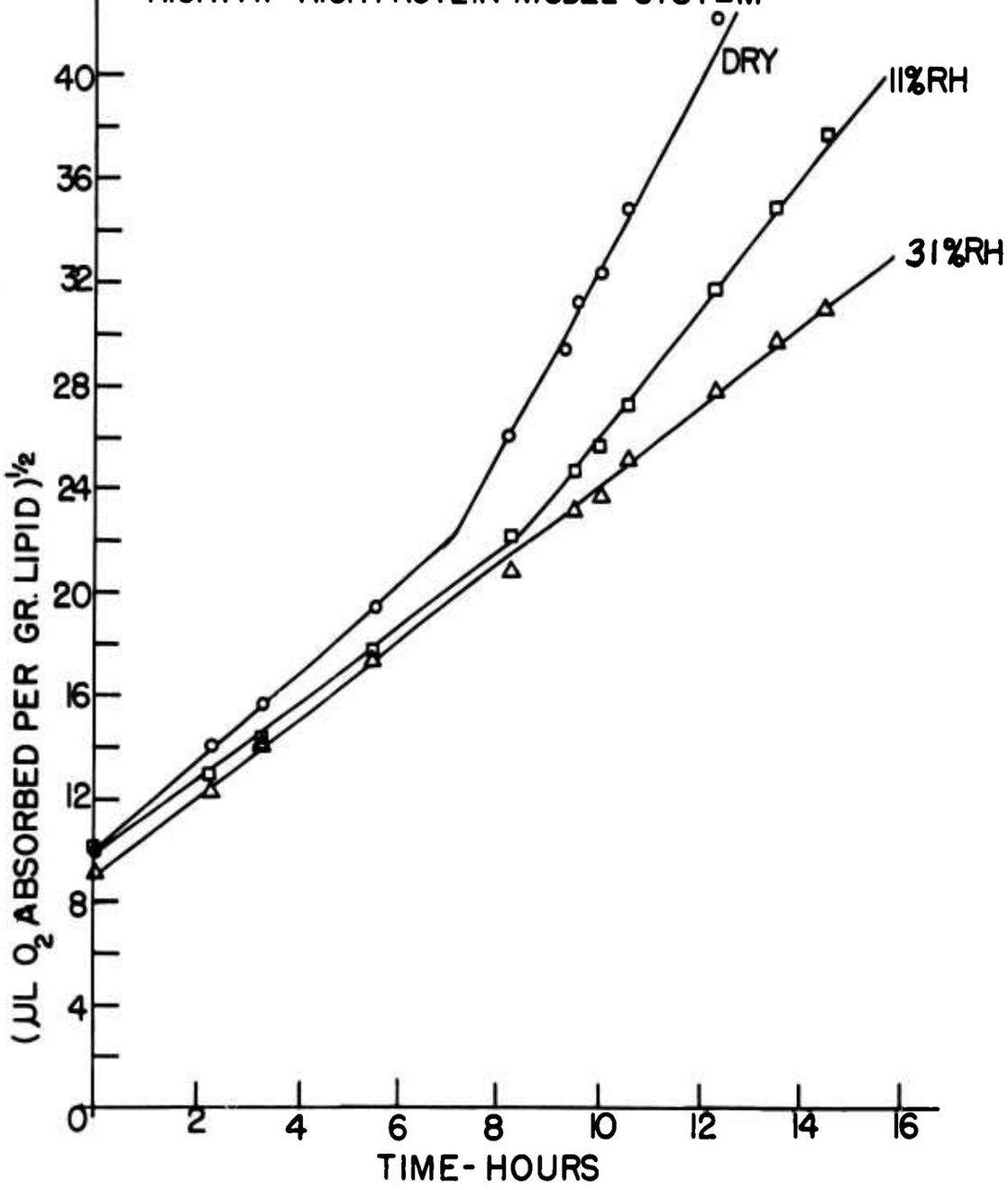


FIGURE 7
RUN 2

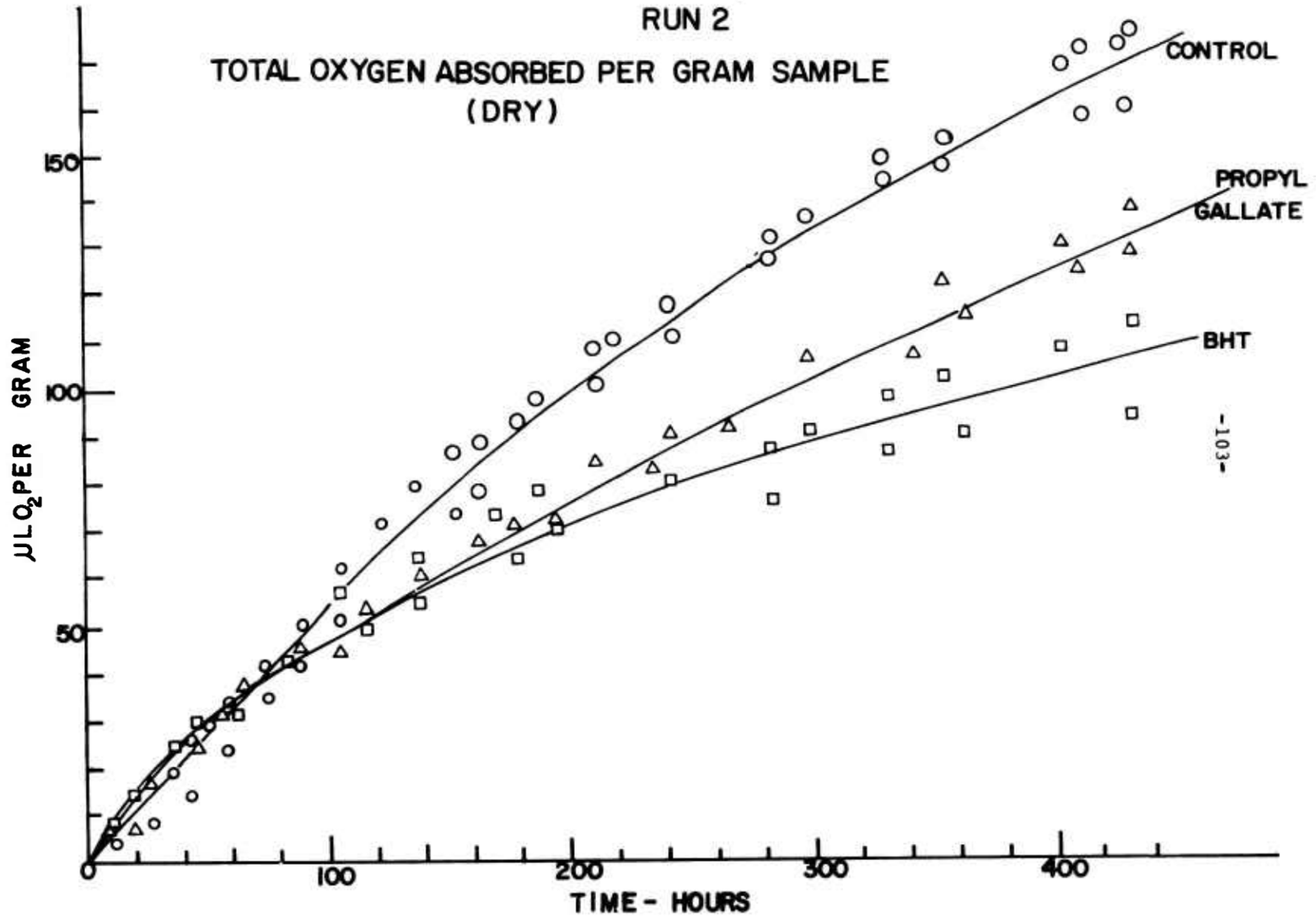
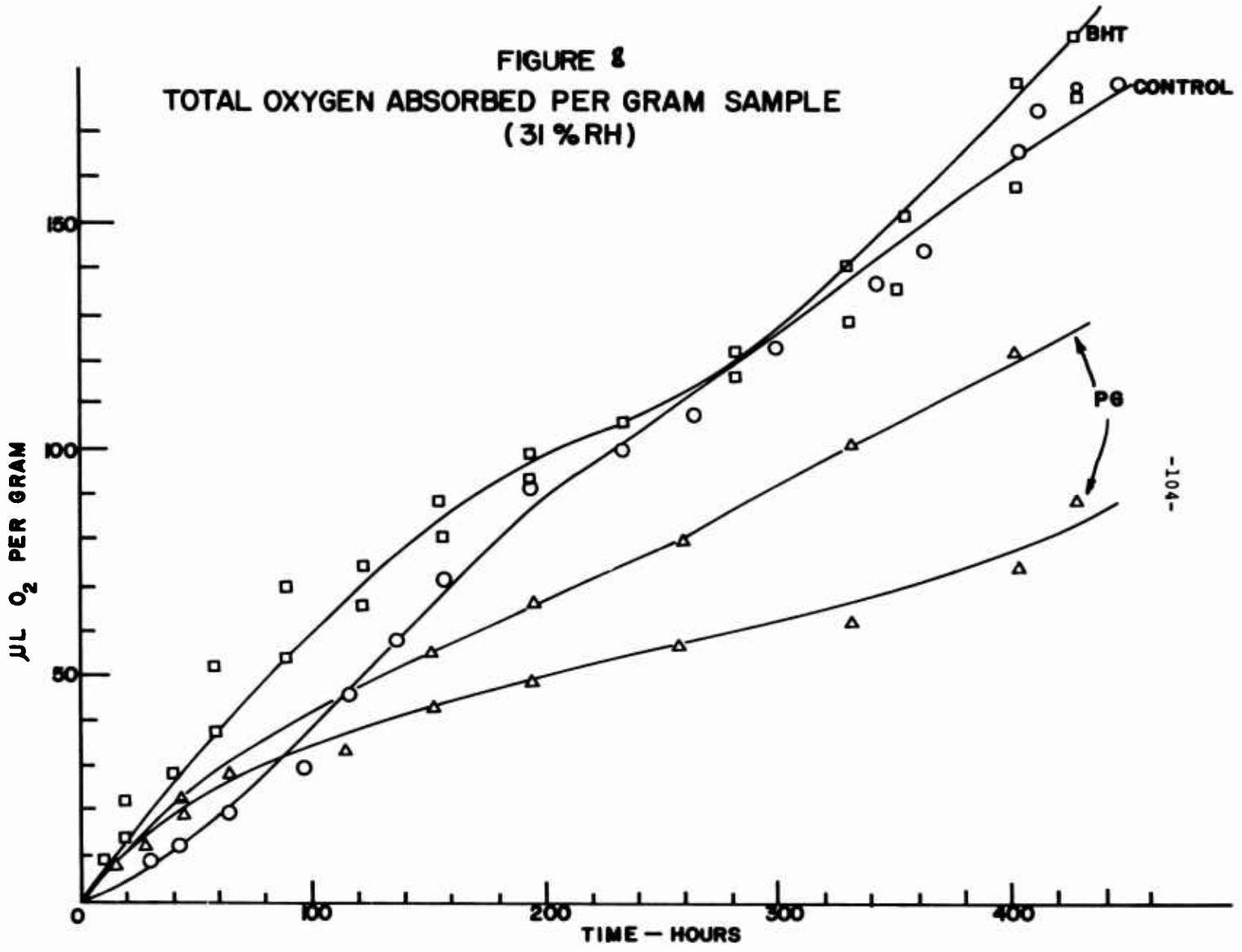


FIGURE 8

TOTAL OXYGEN ABSORBED PER GRAM SAMPLE
(31 % RH)



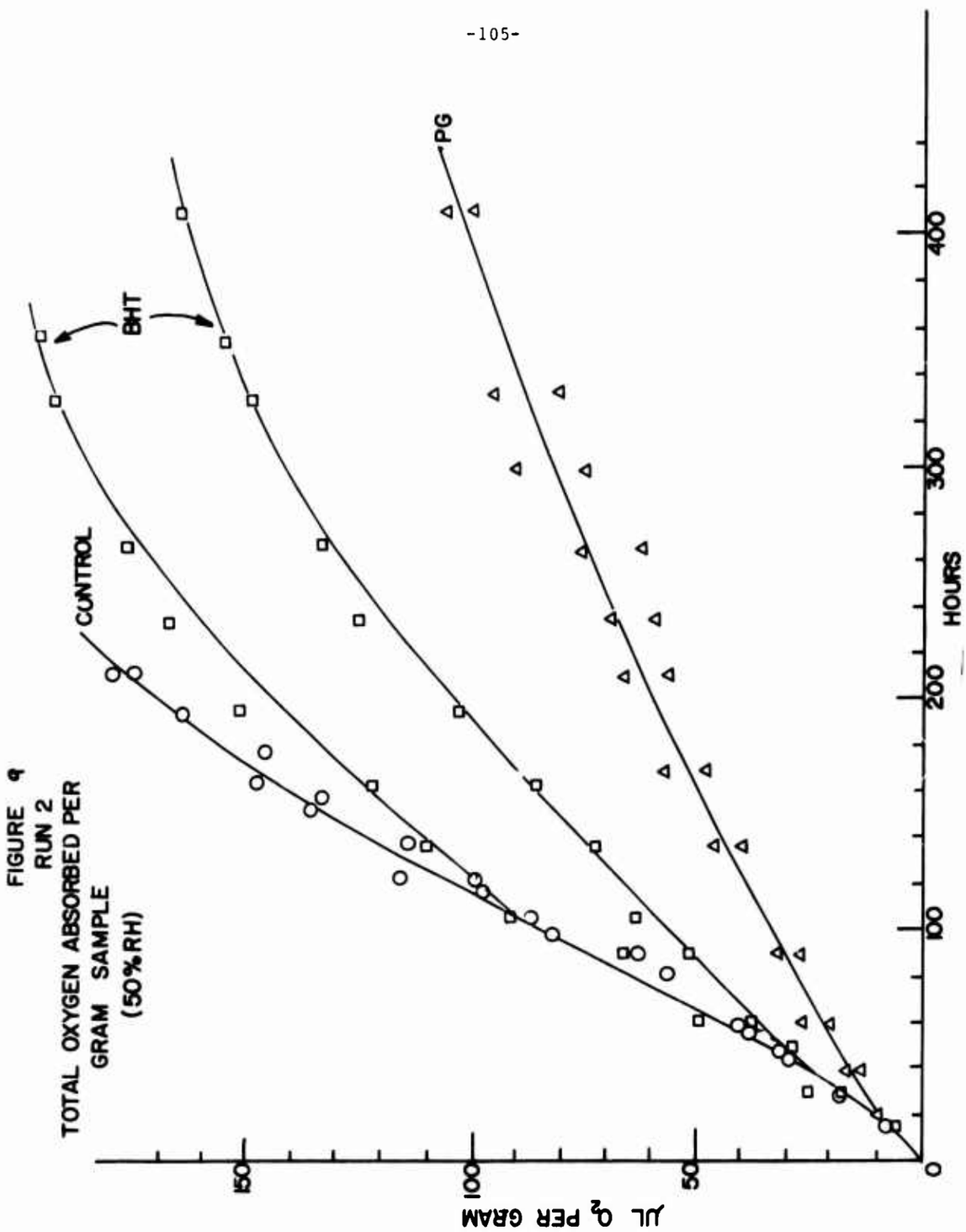


FIGURE 9
RUN 2
TOTAL OXYGEN ABSORBED PER
GRAM SAMPLE
(50% RH)
CONTROL
BHT
PG
0
50
100
150
200
300
400
HOURS
μL O2 PER GRAM

FIGURE 10

TOTAL OXYGEN ABSORBED PER GRAM SAMPLE

RUN 4 CONTROL MODEL

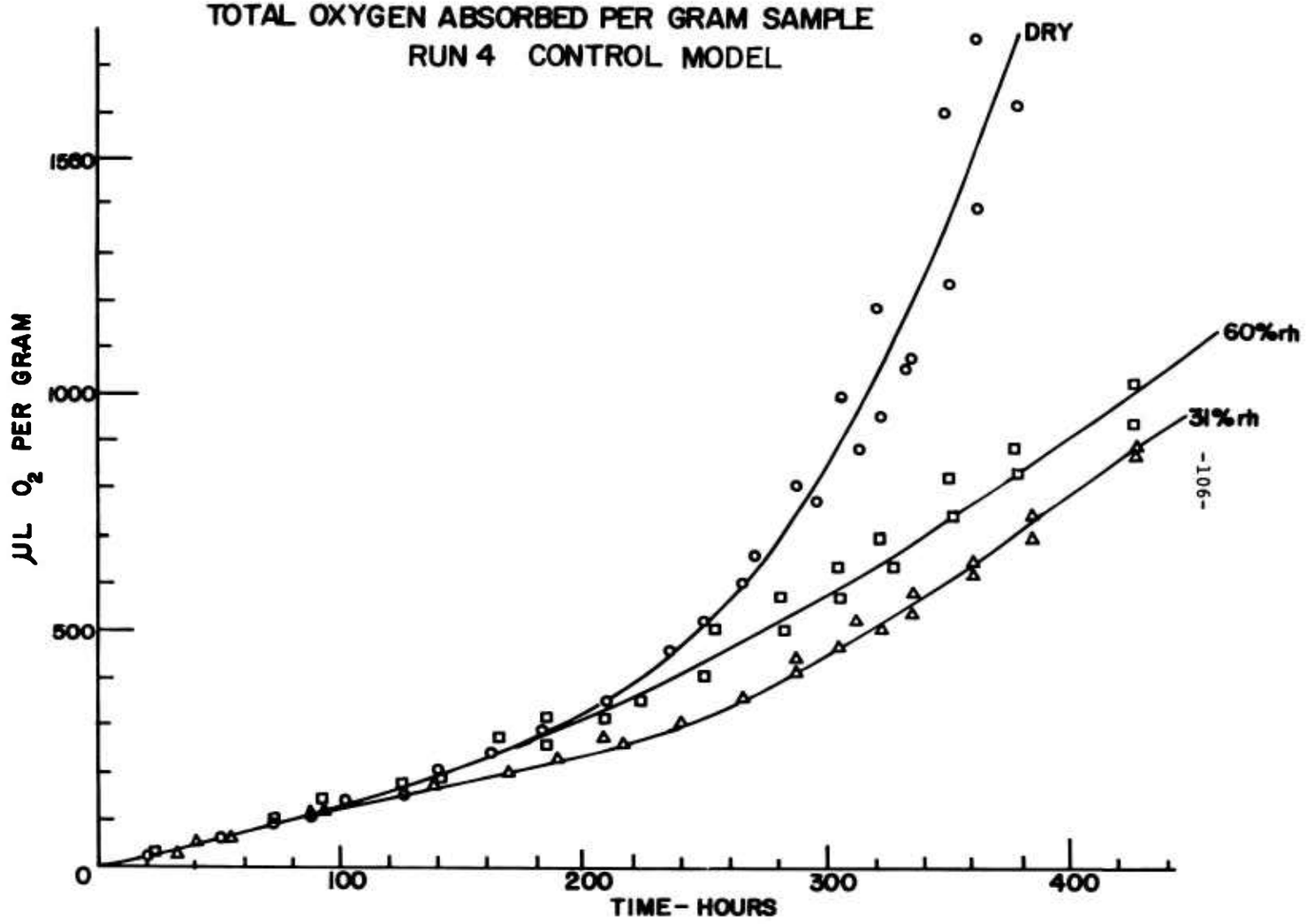


FIGURE II

TOTAL OXYGEN ABSORBED PER GRAM SAMPLE

RUN 4 BHT MODEL

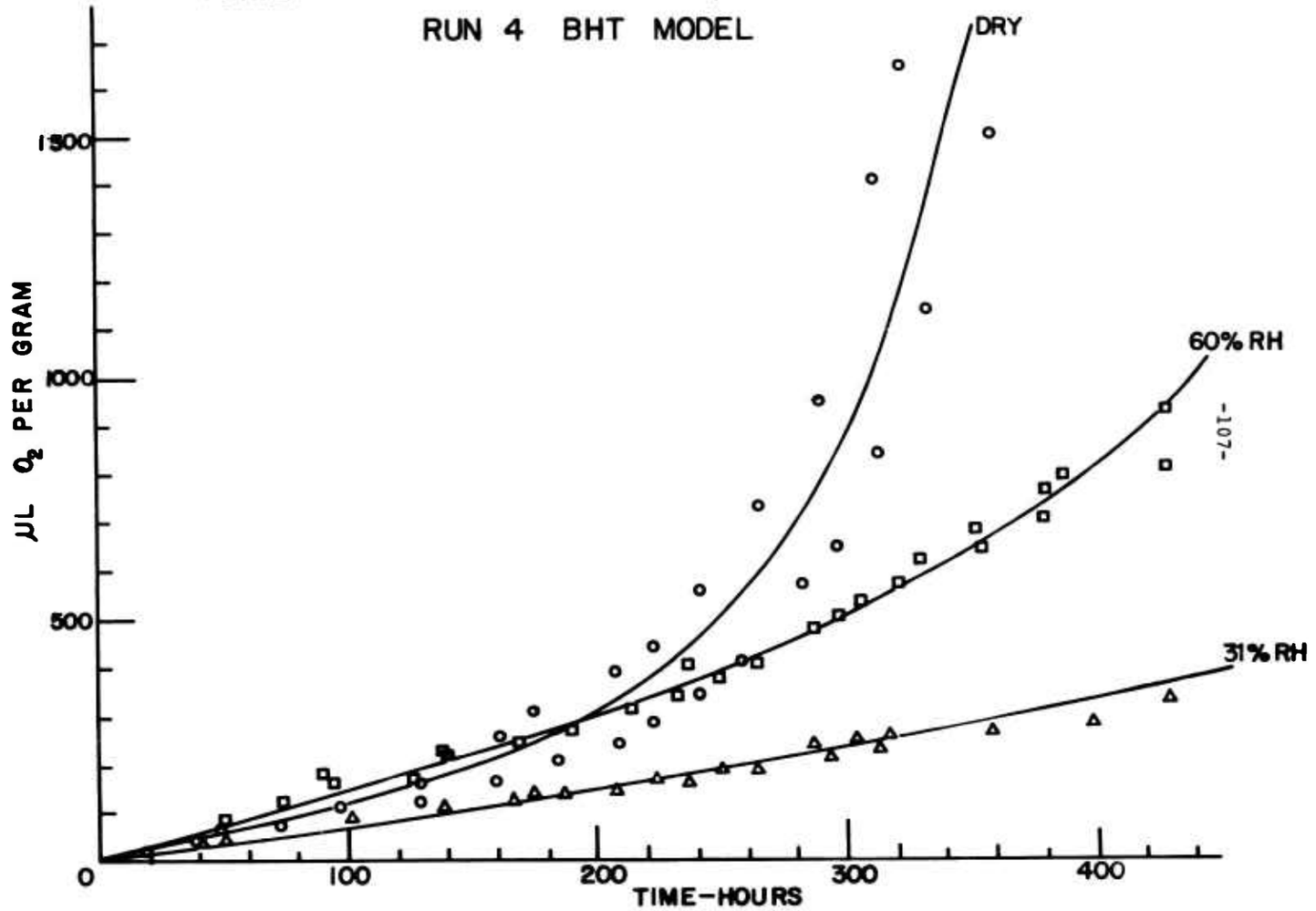


FIGURE 12
TOTAL OXYGEN ABSORBED PER GRAM SAMPLE
RUN 4 PG MODEL

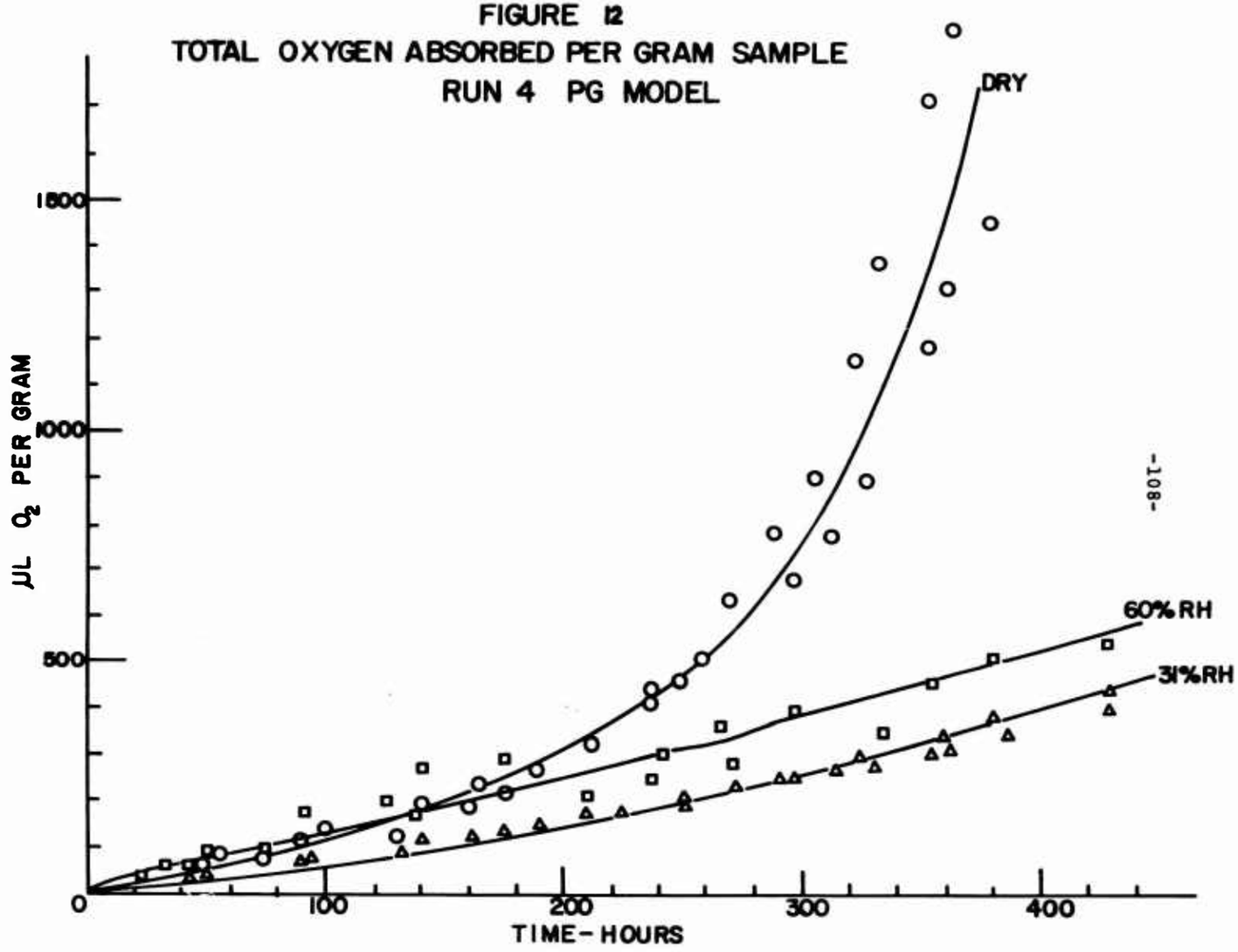


FIGURE 18

TOTAL OXYGEN ABSORBED PER GRAM SAMPLE
RUN 5 CONTROL MODEL

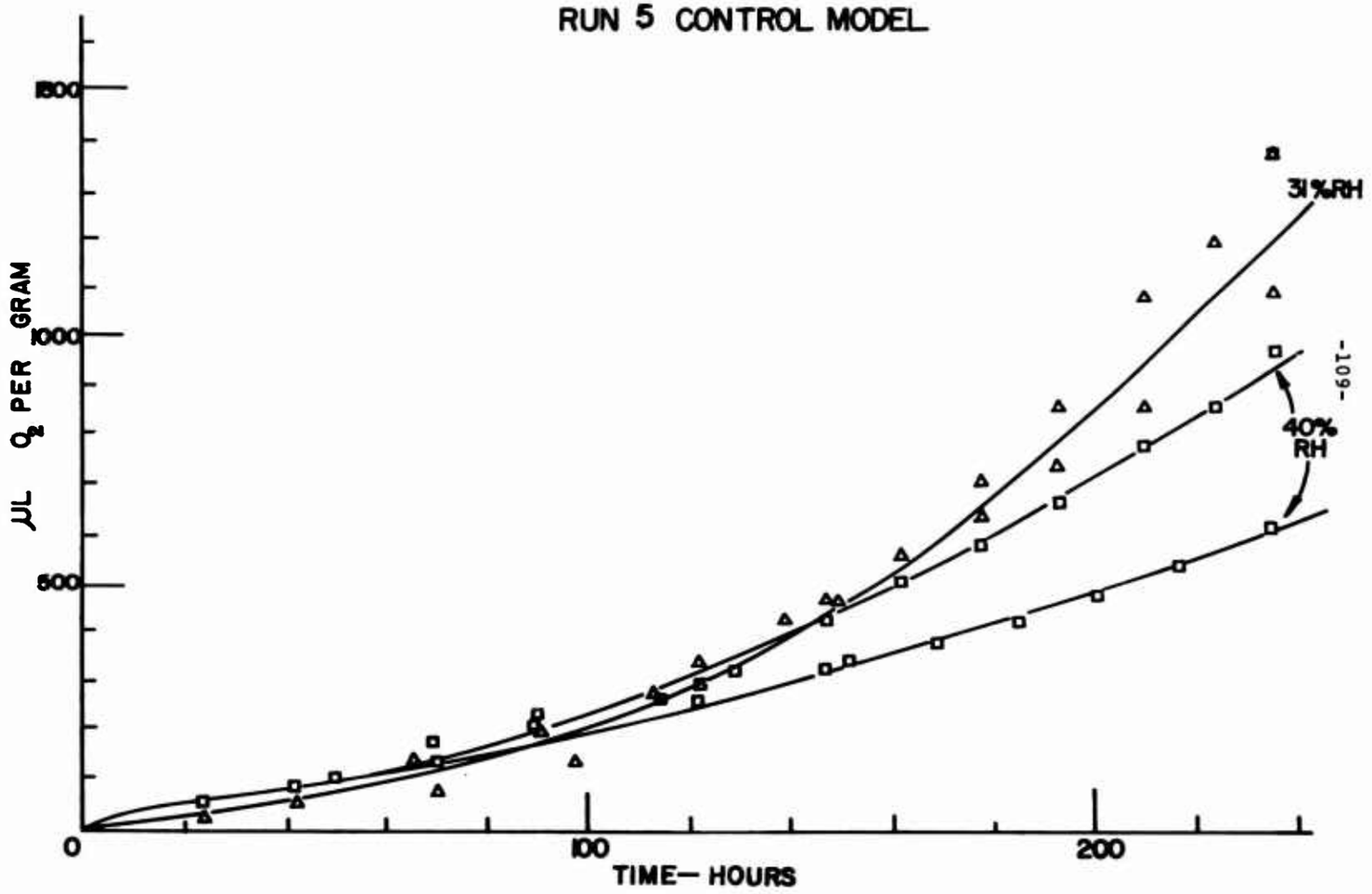


FIGURE 14

TOTAL OXYGEN ABSORBED PER GRAM SAMPLE

RUN 5 COBALT MODEL

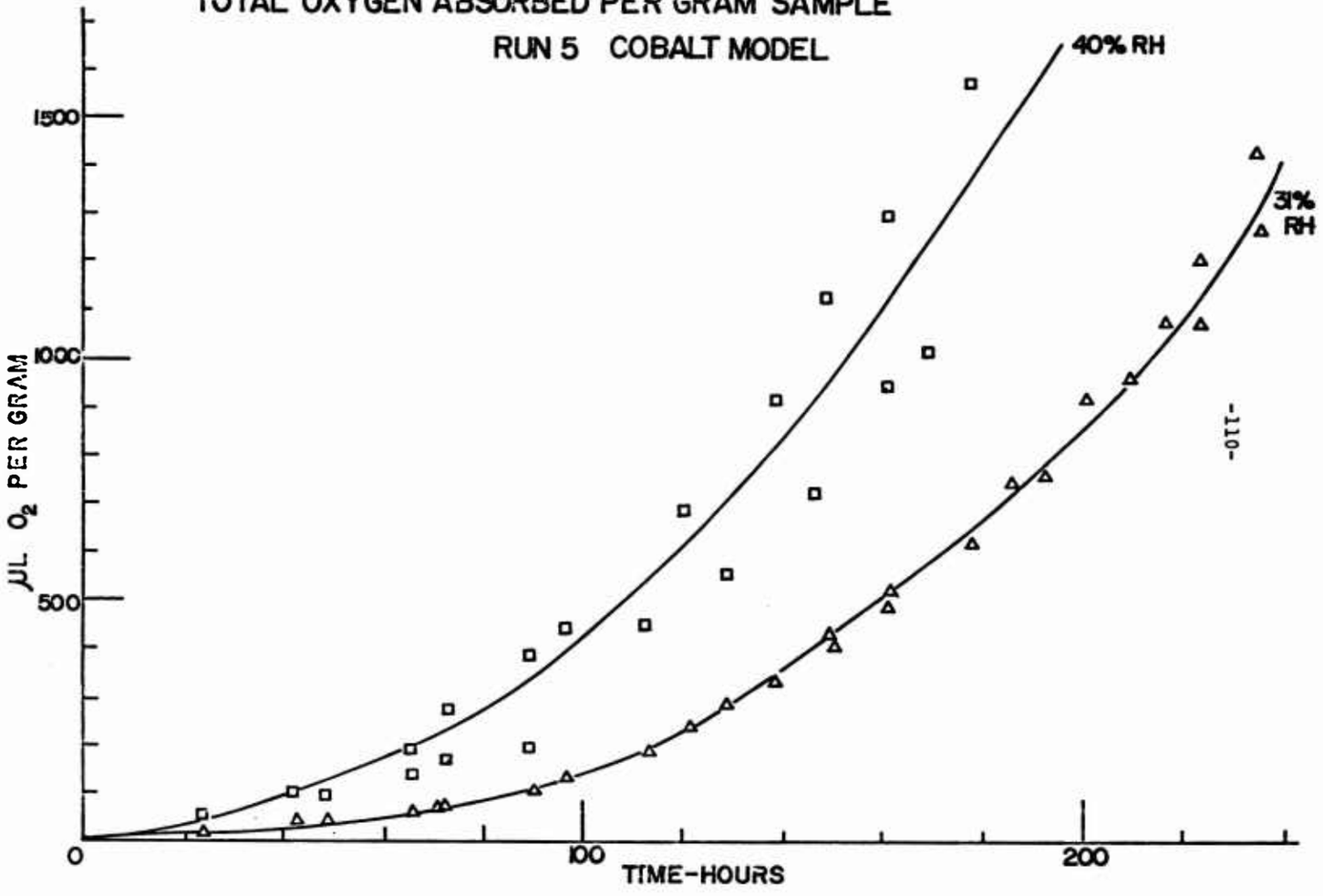


FIGURE 15

TOTAL OXYGEN ABSORBED PER GRAM SAMPLE

RUN 5 HISTIDINE MODEL

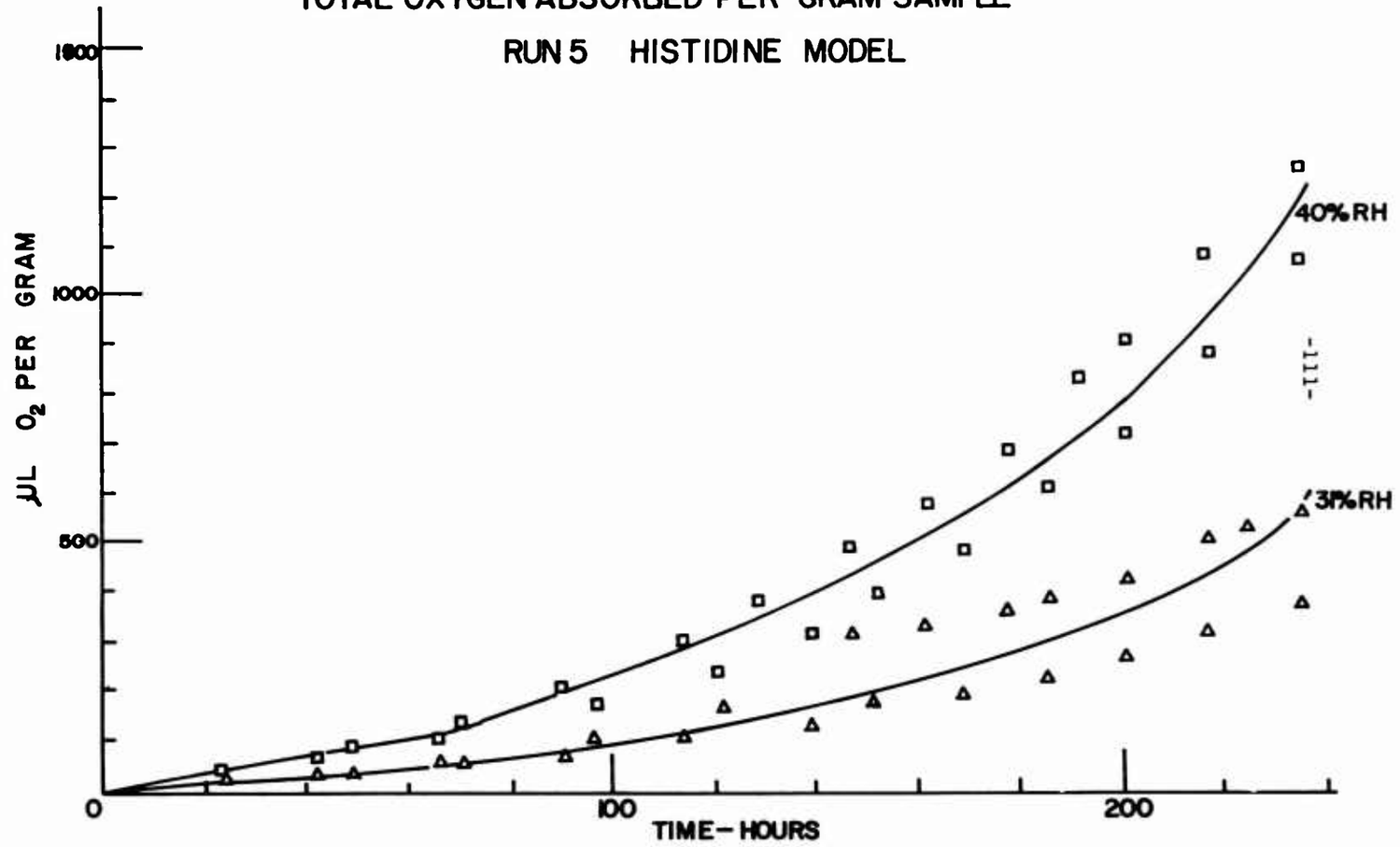


FIGURE 16

TOTAL OXYGEN ABSORBED PER GRAM SAMPLE

RUN 5 COBALT & HISTIDINE MODEL

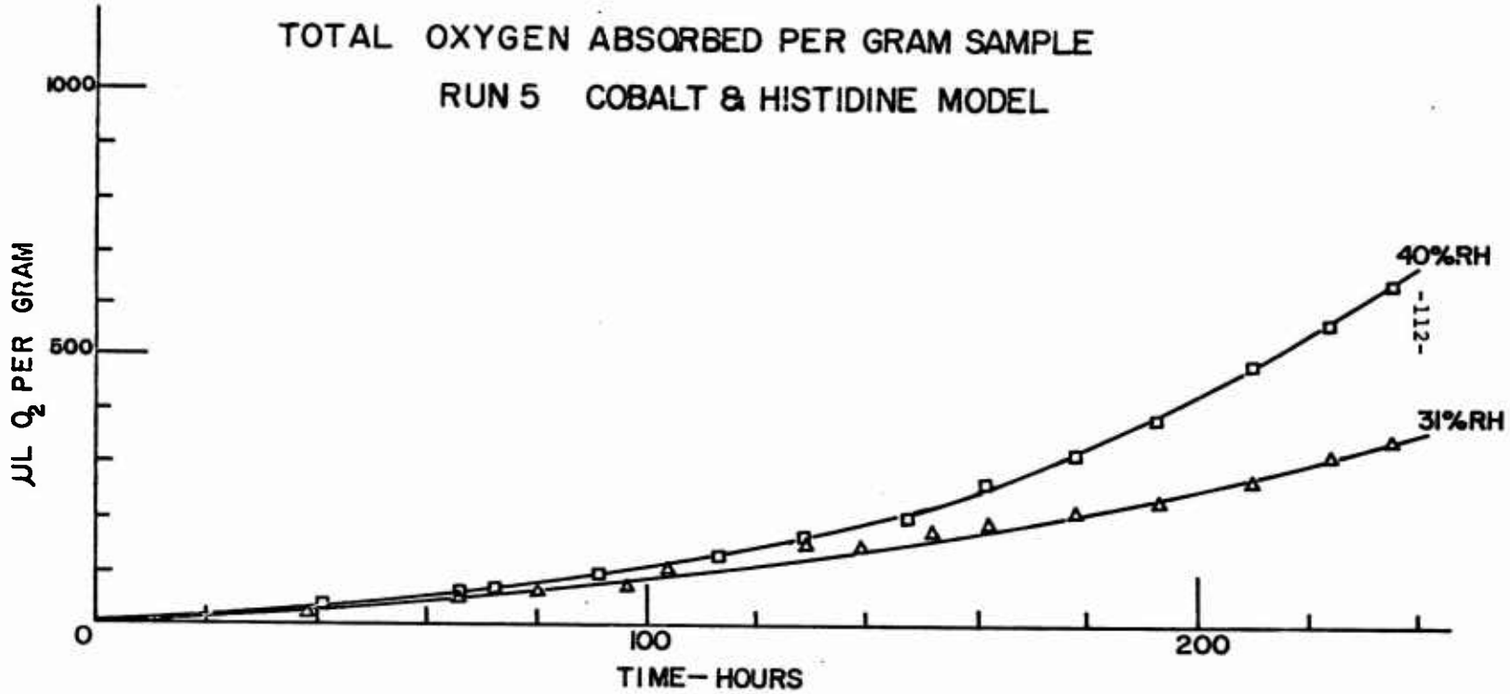


FIGURE 17

TOTAL OXYGEN ABSORBED PER GRAM SAMPLE

RUN 5 EDTA MODEL

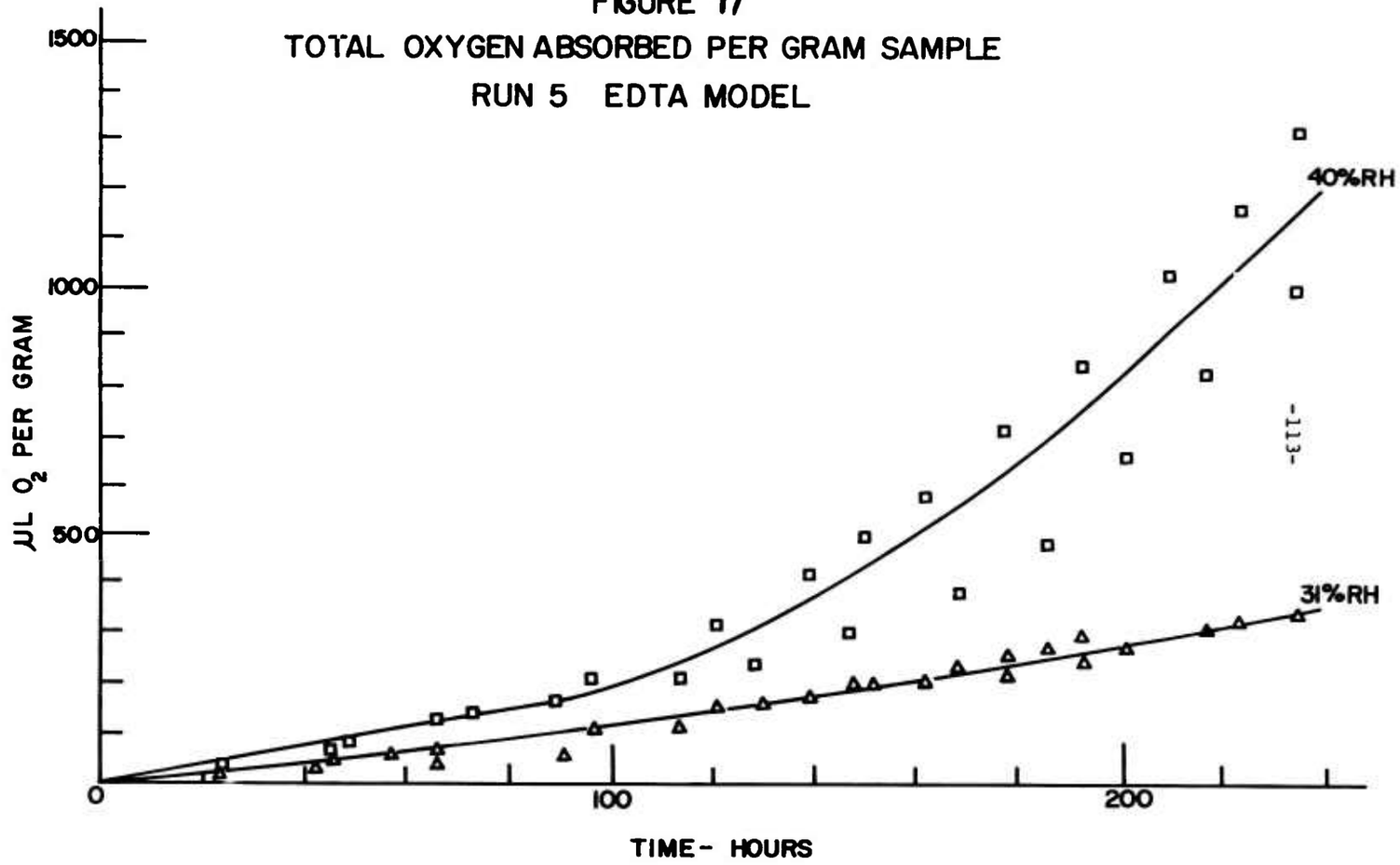
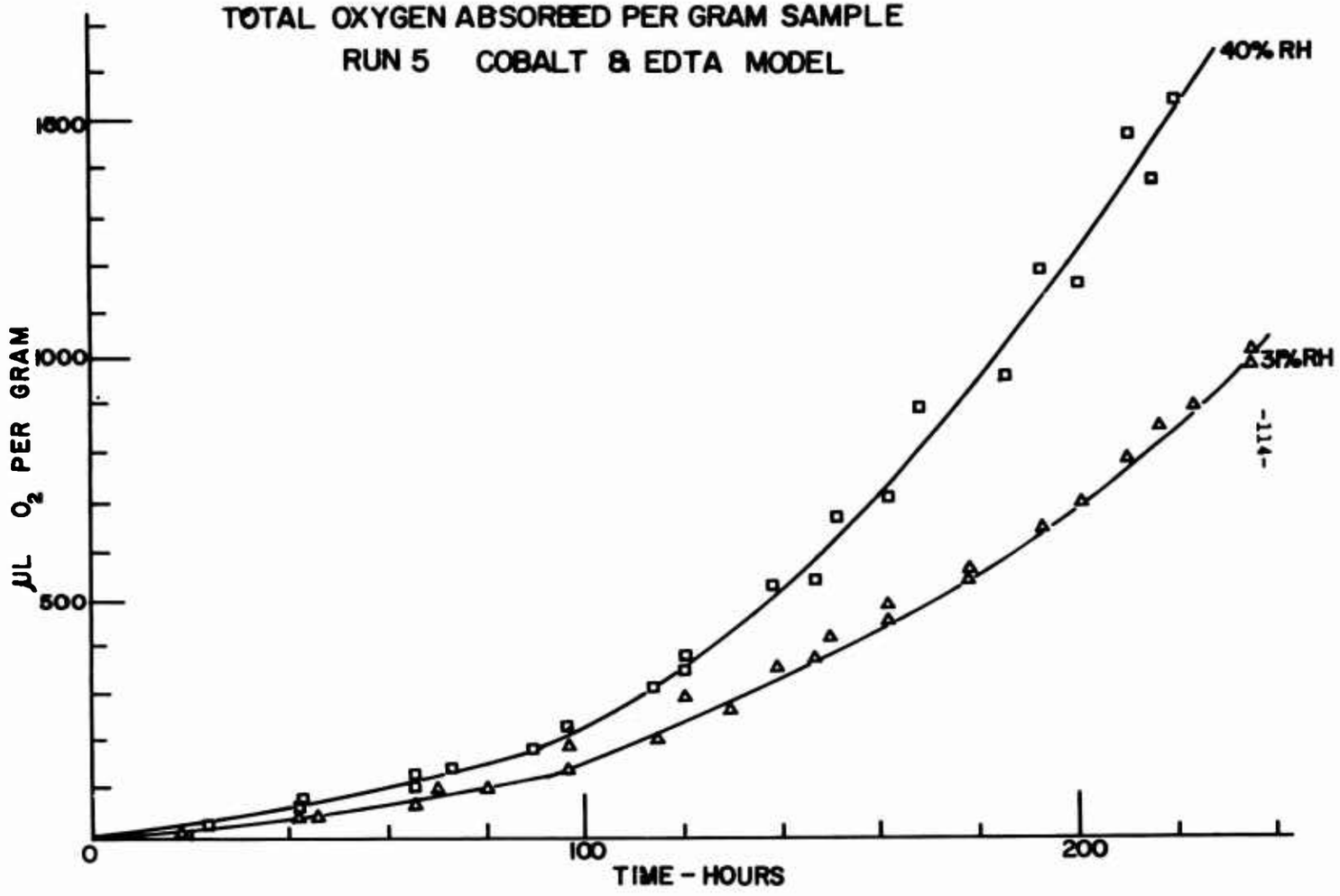


FIGURE 18

TOTAL OXYGEN ABSORBED PER GRAM SAMPLE

RUN 5 COBALT & EDTA MODEL



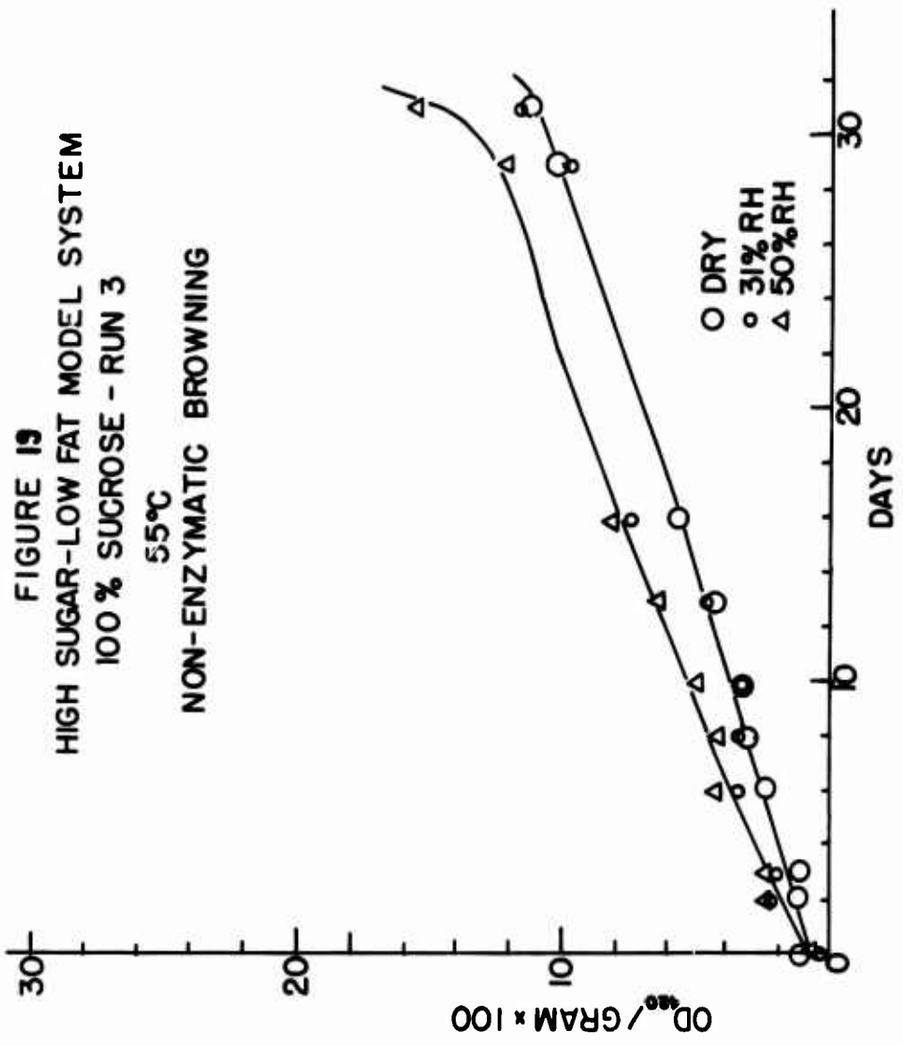


FIGURE 19
HIGH SUGAR-LOW FAT MODEL SYSTEM
100% SUCROSE - RUN 3
55°C
NON-ENZYMATIC BROWNING
O DRY
o 31% RH
Δ 50% RH
OD420/GRAM × 100
DAYS
0
10
20
30

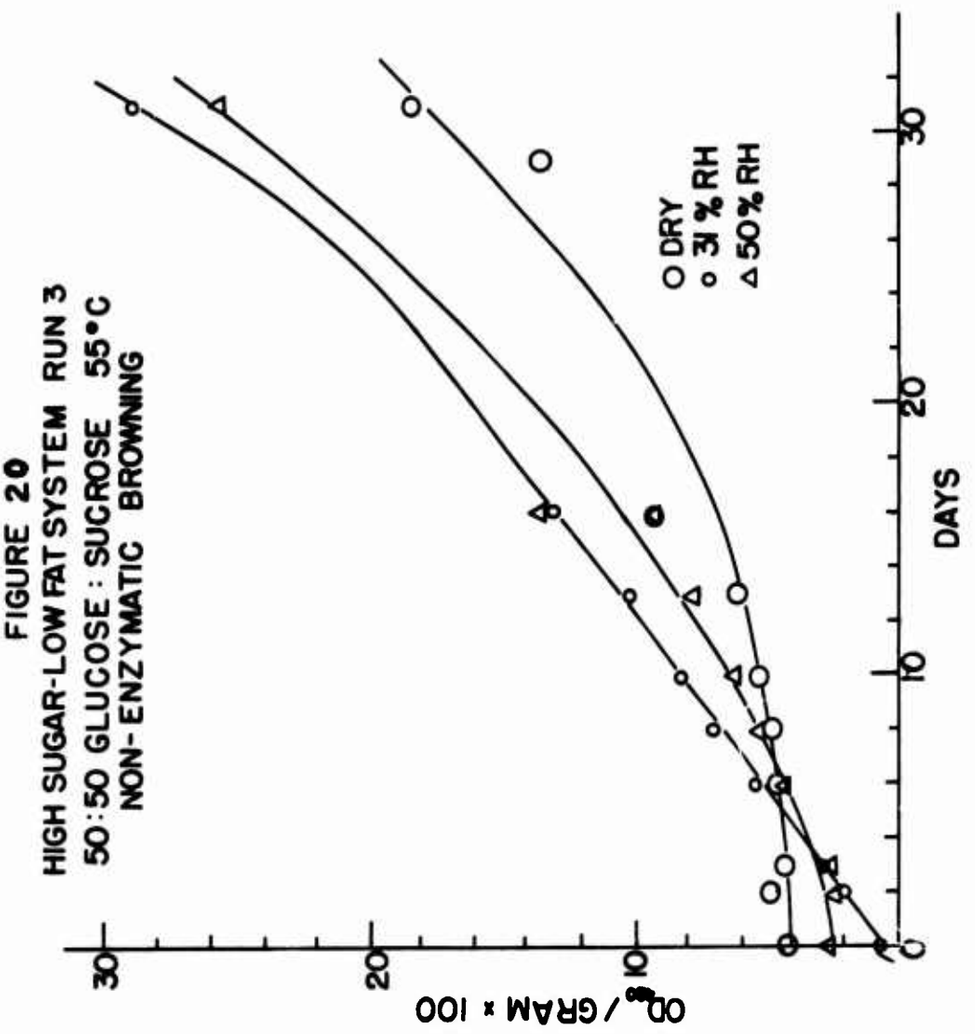
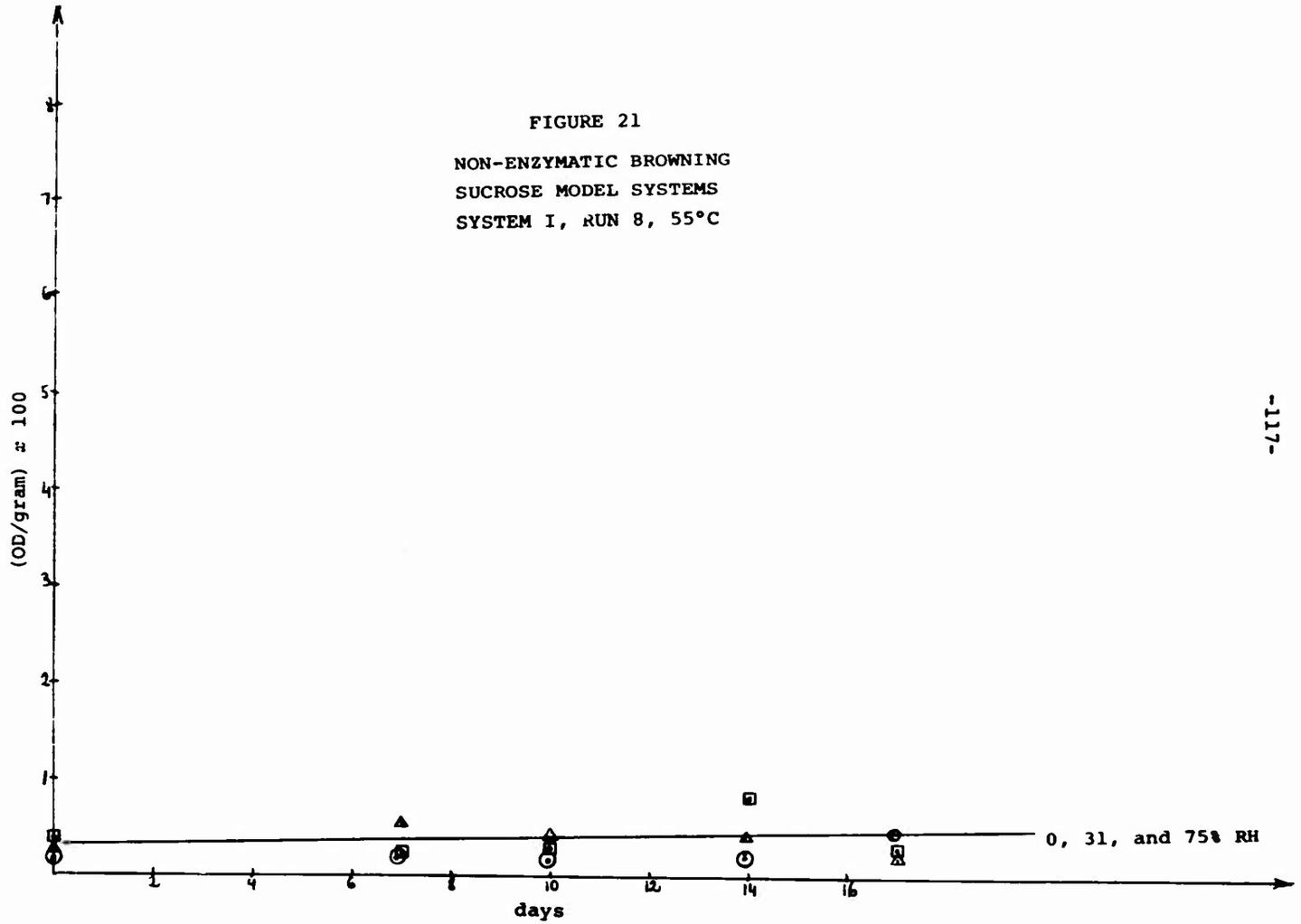


FIGURE 20
HIGH SUGAR-LOW FAT SYSTEM RUN 3
50:50 GLUCOSE : SUCROSE 55°C
NON-ENZYMATIC BROWNING
O DRY
o 31 % RH
Δ 50 % RH
OD 490 / GRAM x 100
DAYS
0
10
20
30

FIGURE 21

NON-ENZYMATIC BROWNING
SUCROSE MODEL SYSTEMS
SYSTEM I, RUN 8, 55°C



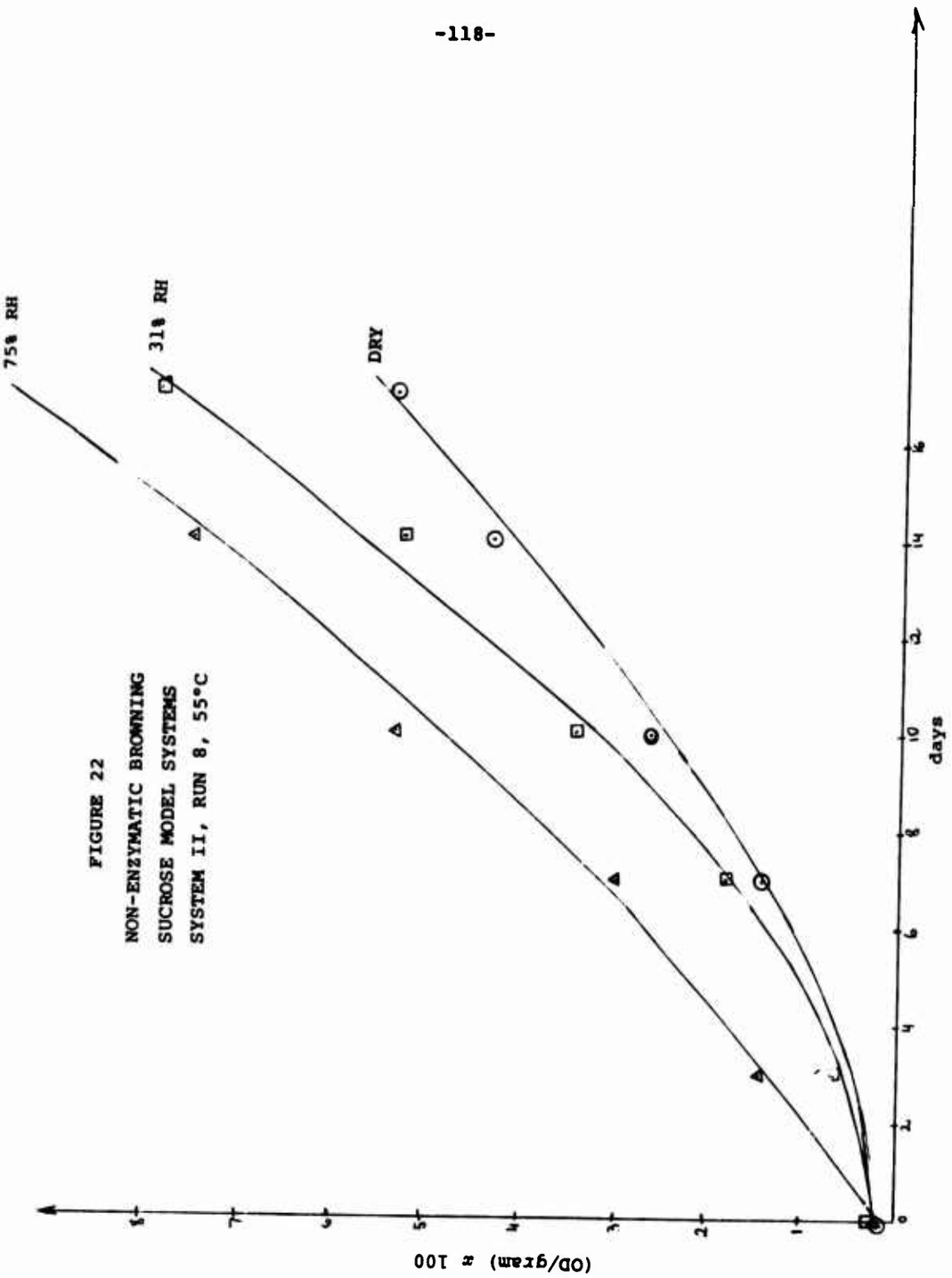


FIGURE 22
NON-ENZYMATIC BROWNING
SUCROSE MODEL SYSTEMS
SYSTEM II, RUN 8, 55°C

FIGURE 23

NON-ENZYMATIC BROWNING
SUCROSE MODEL SYSTEMS
SYSTEM III, RUN 8, 55°C

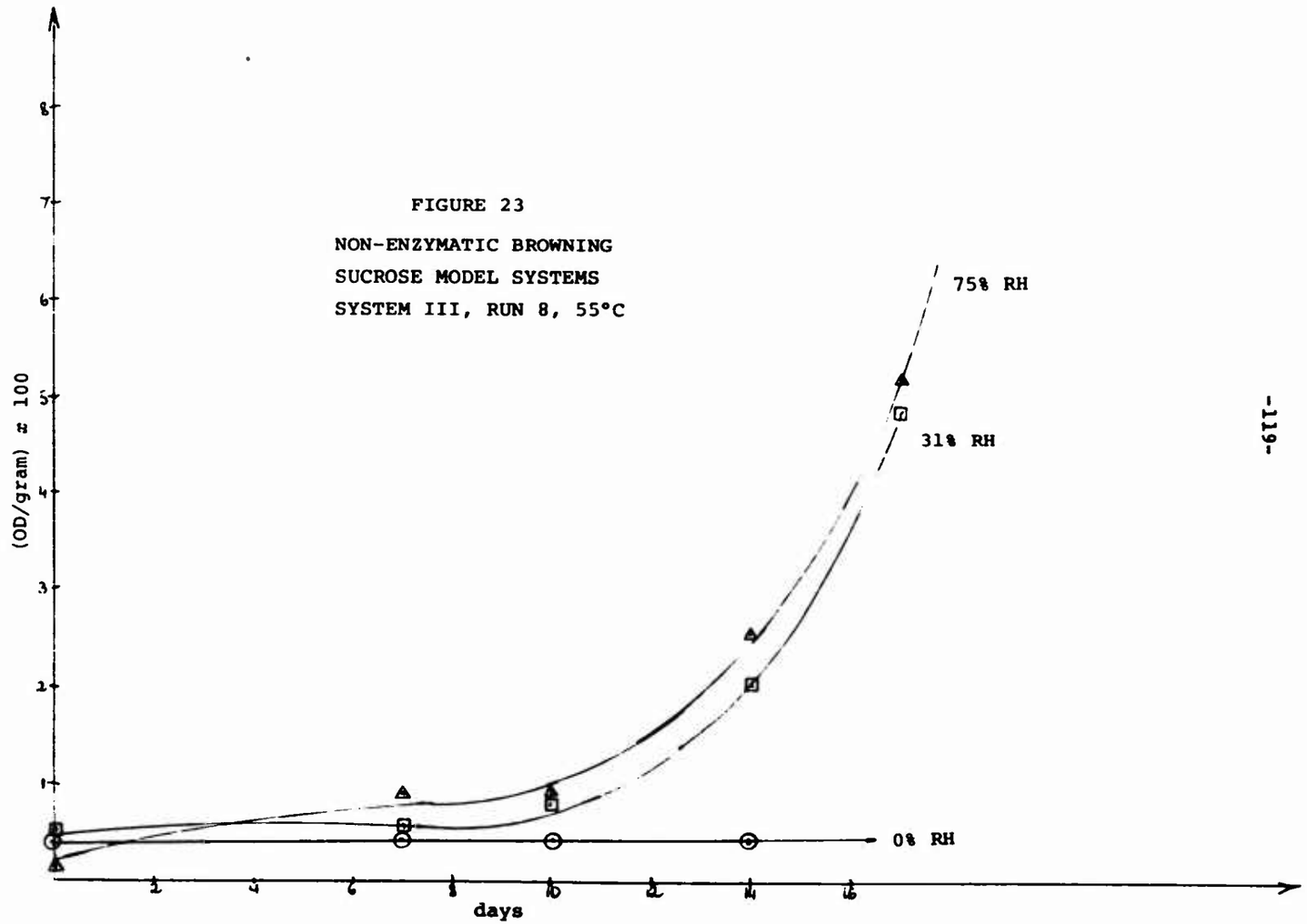


FIGURE 24

PERCENT SUCROSE HYDROLIZED

RUN 10

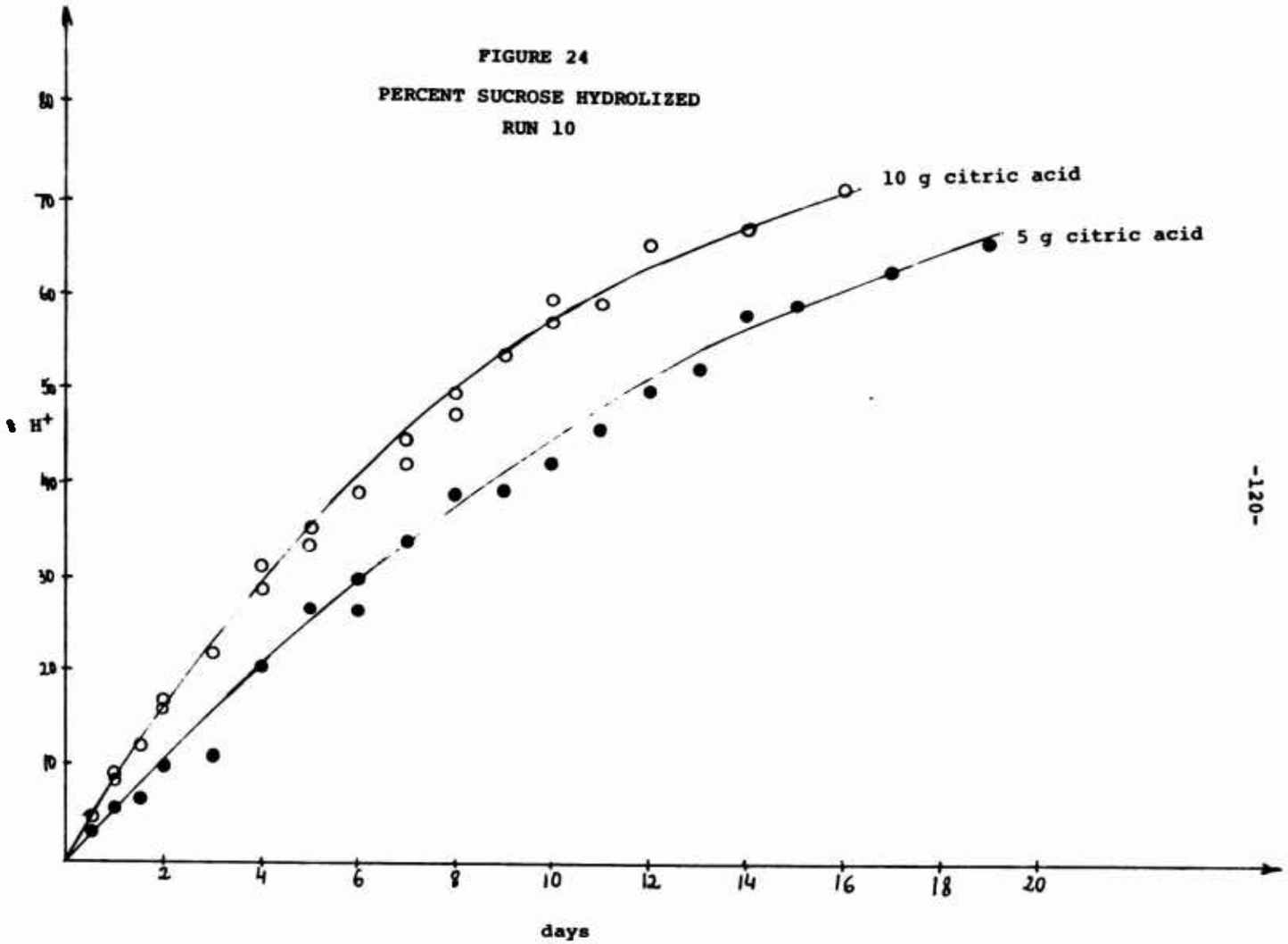
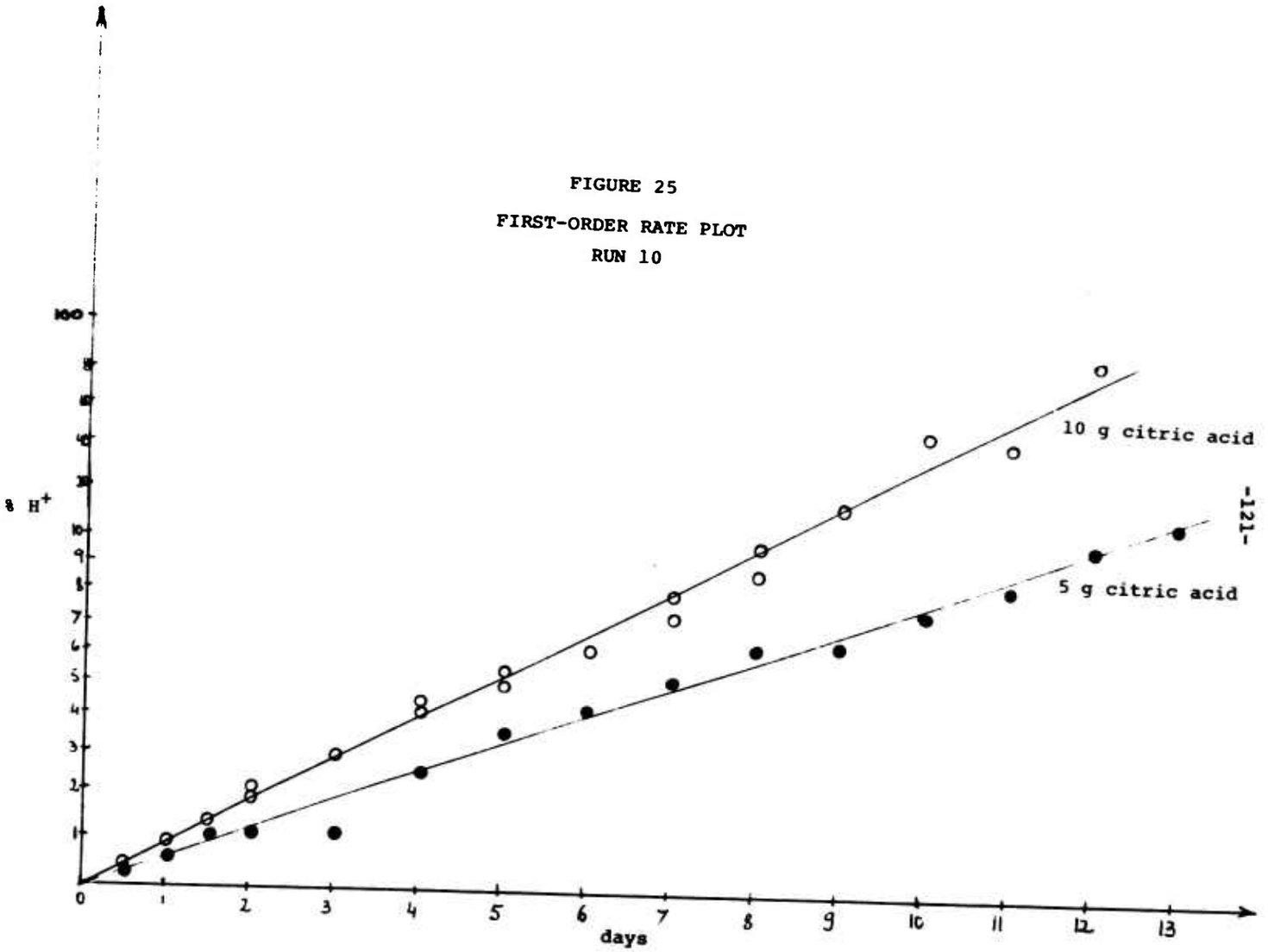


FIGURE 25

FIRST-ORDER RATE PLOT

RUN 10



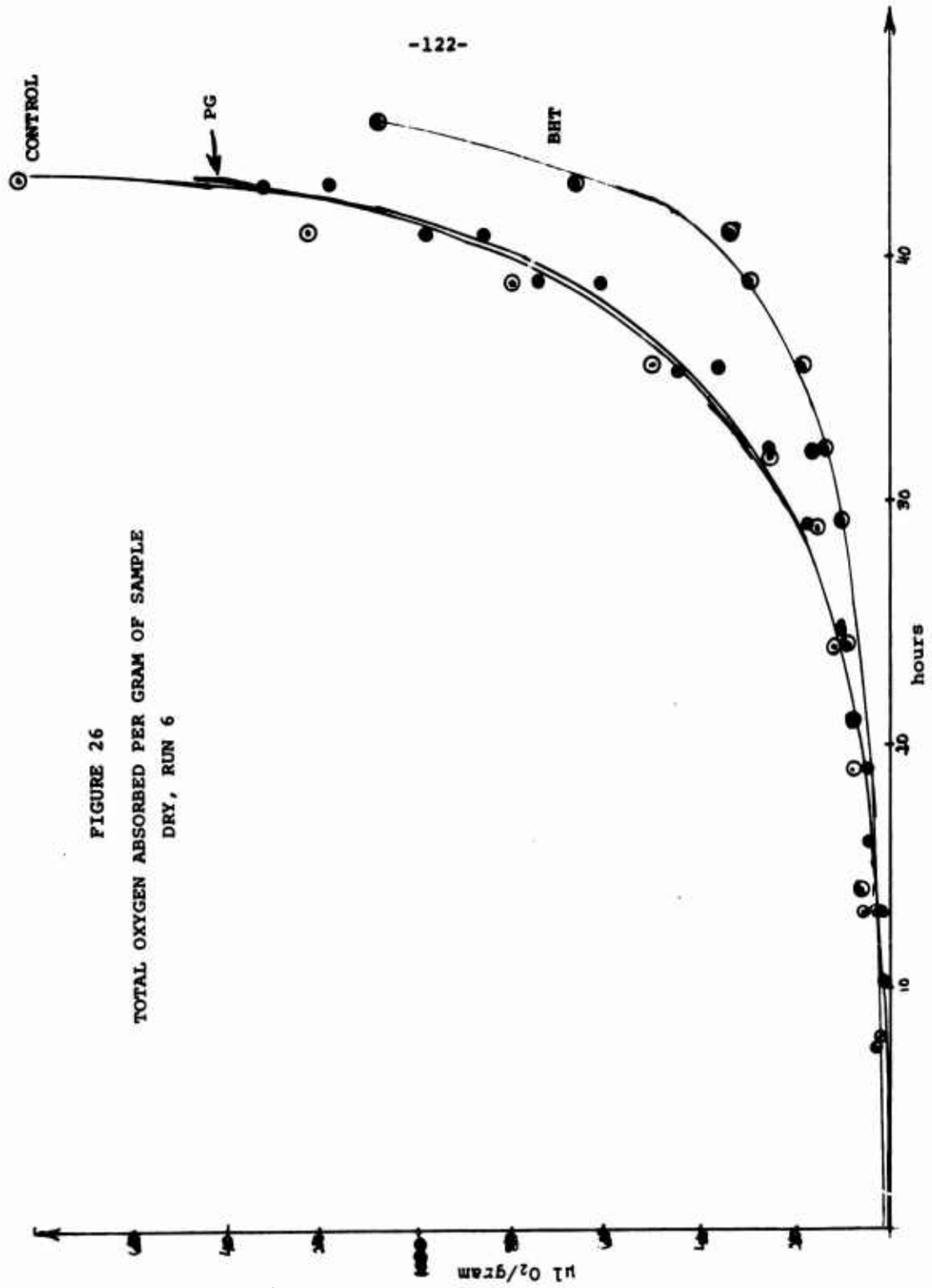


FIGURE 26

TOTAL OXYGEN ABSORBED PER GRAM OF SAMPLE

DRY, RUN 6

FIGURE 27

TOTAL OXYGEN ABSORBED PER GRAM OF SAMPLE

11% RH, RUN 6

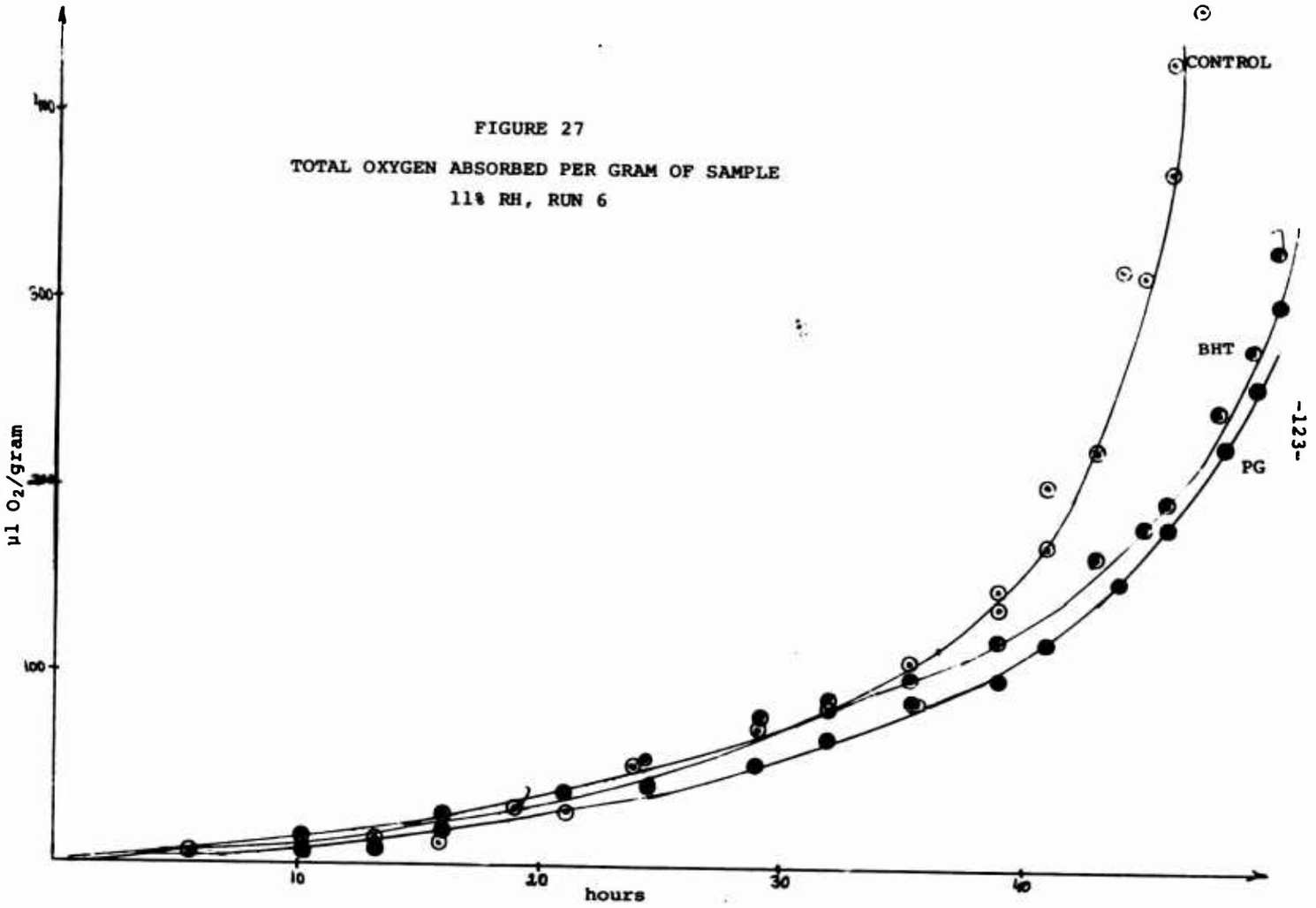


FIGURE 28

TOTAL OXYGEN ABSORBED PER GRAM OF SAMPLE
31% RH, RUN 6

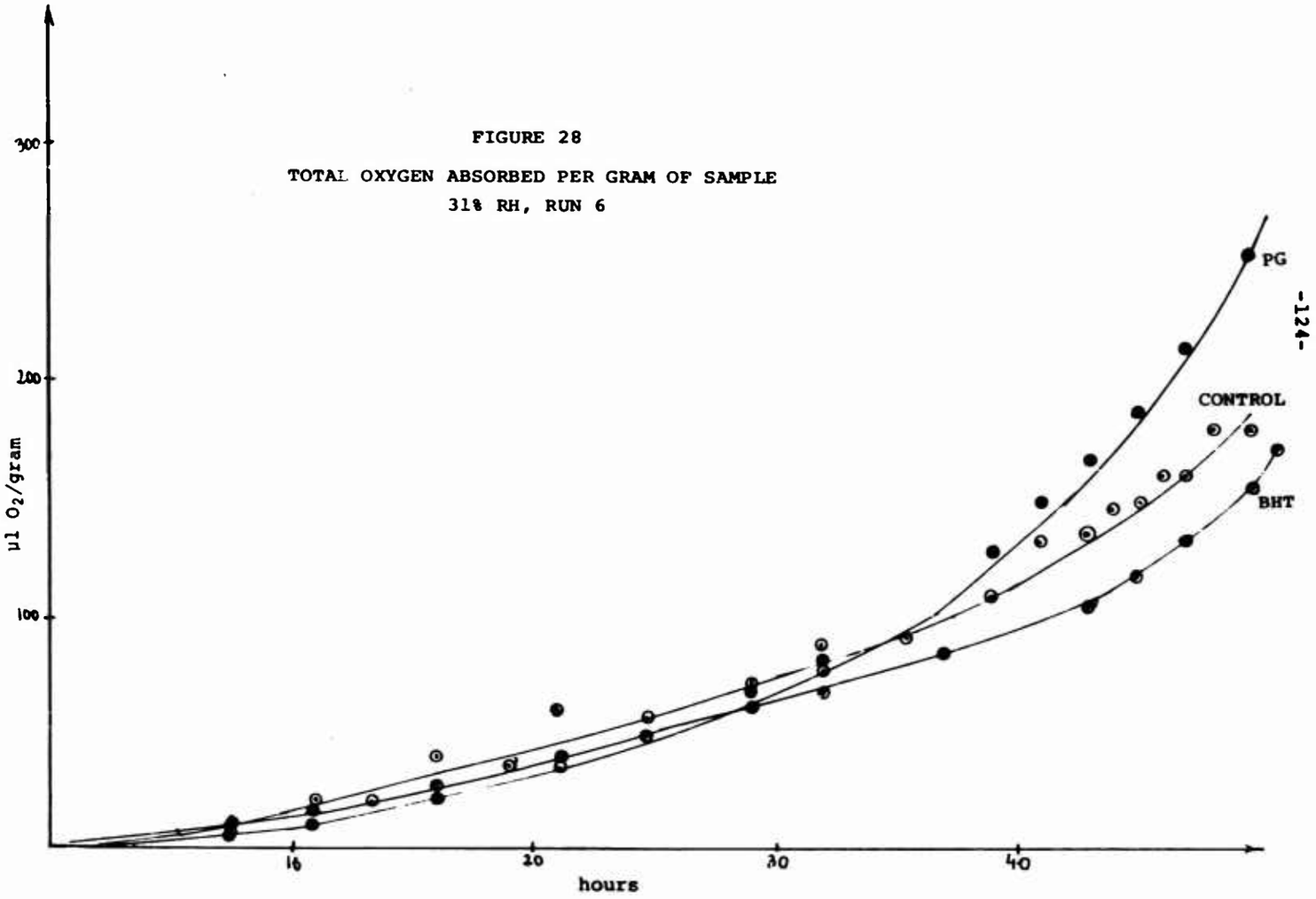
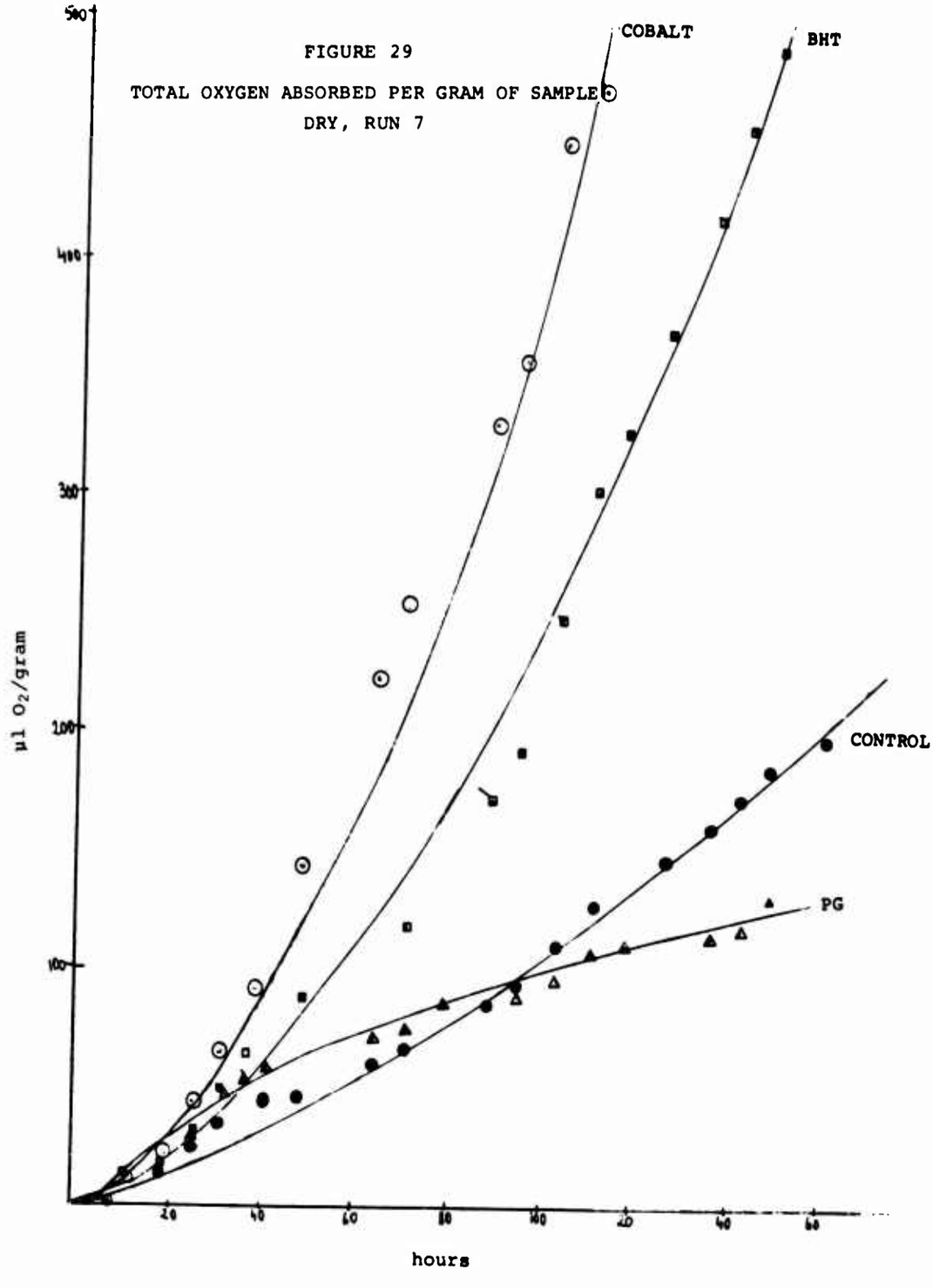


FIGURE 29

TOTAL OXYGEN ABSORBED PER GRAM OF SAMPLE
DRY, RUN 7



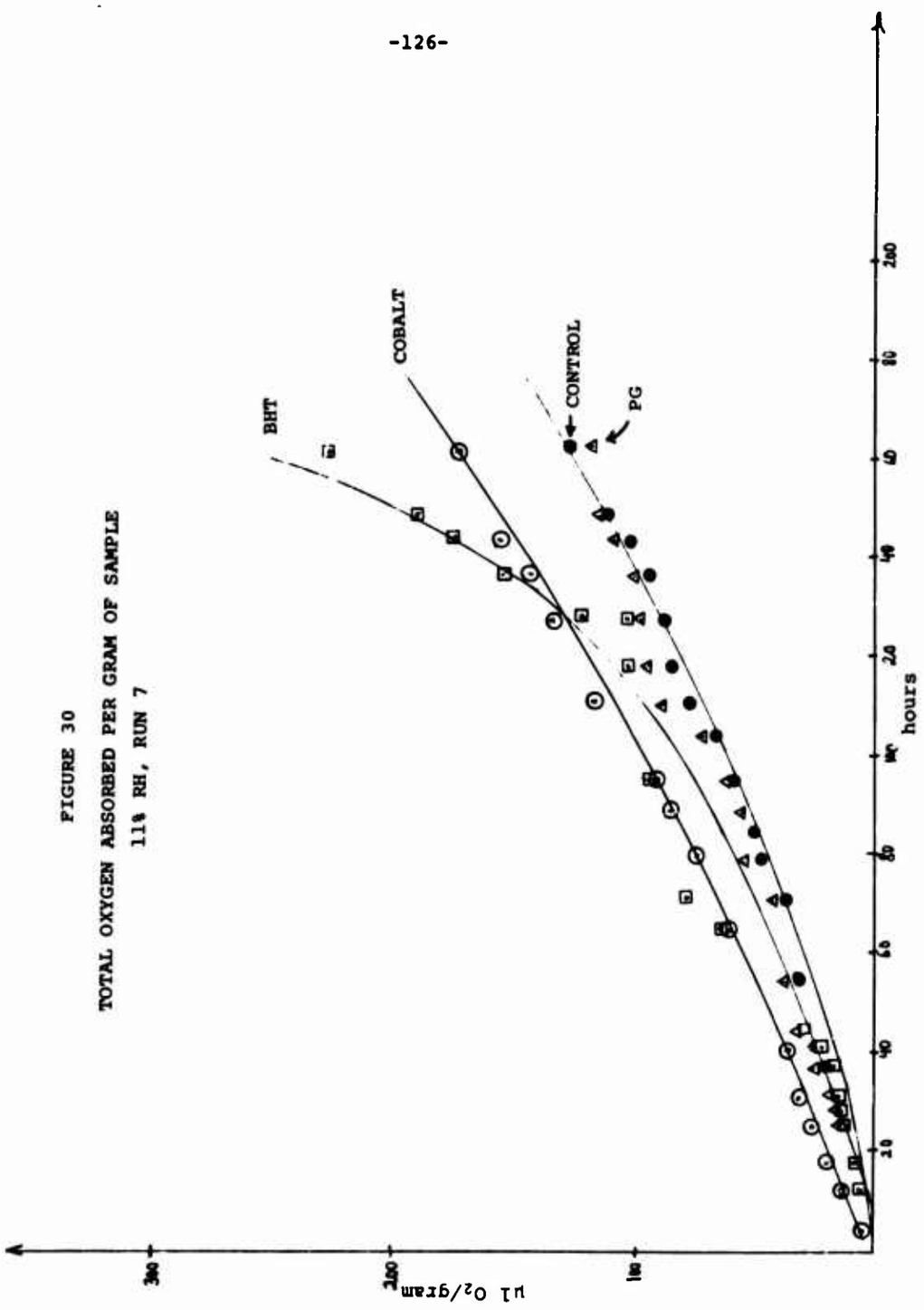


FIGURE 30

TOTAL OXYGEN ABSORBED PER GRAM OF SAMPLE

11% RH, RUN 7

FIGURE 31

TOTAL OXYGEN ABSORBED PER GRAM OF SAMPLE
31% RH, RUN 7

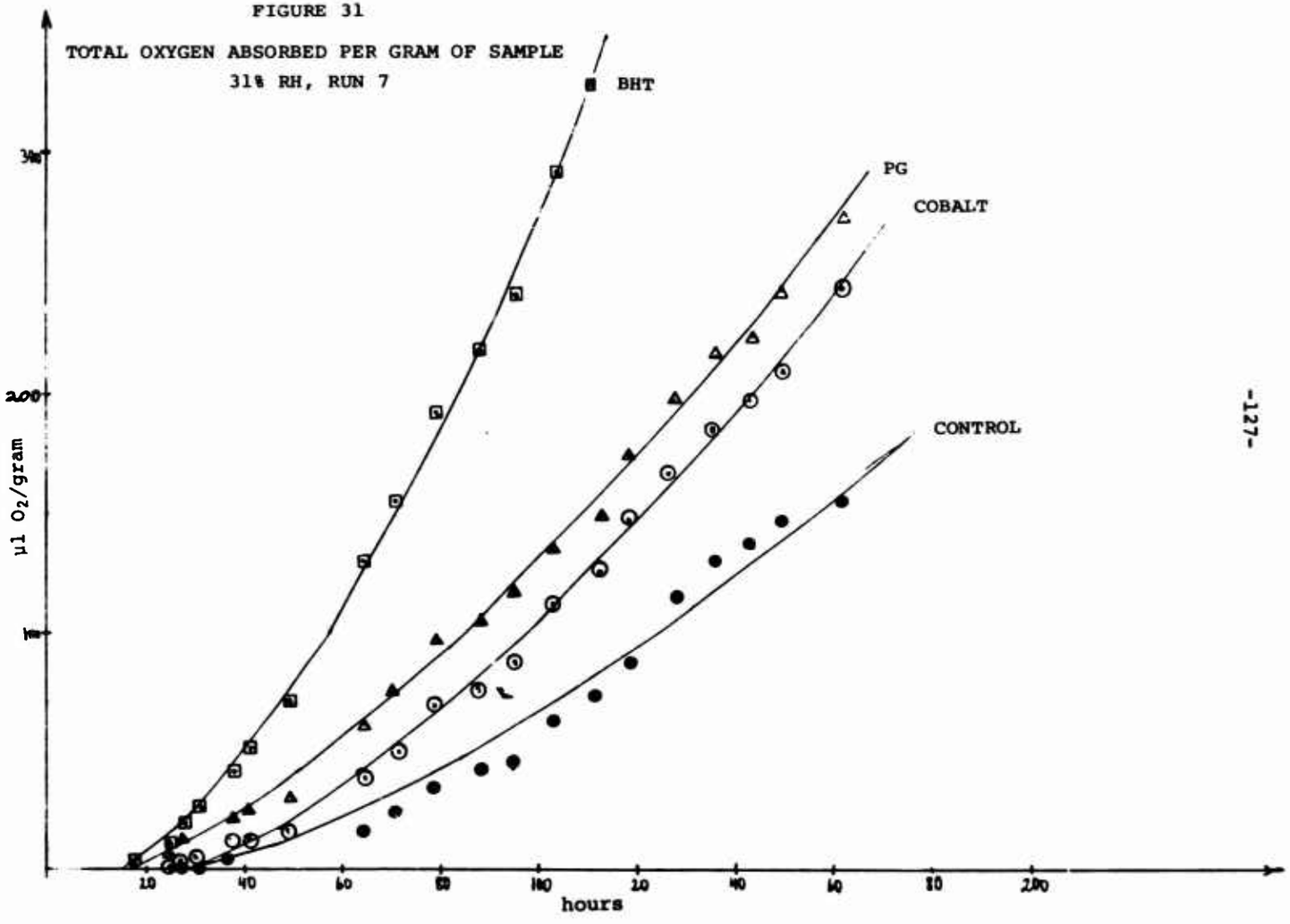


FIGURE 32

SQUARE ROOT PLOT

PG SAMPLE, RUN 6

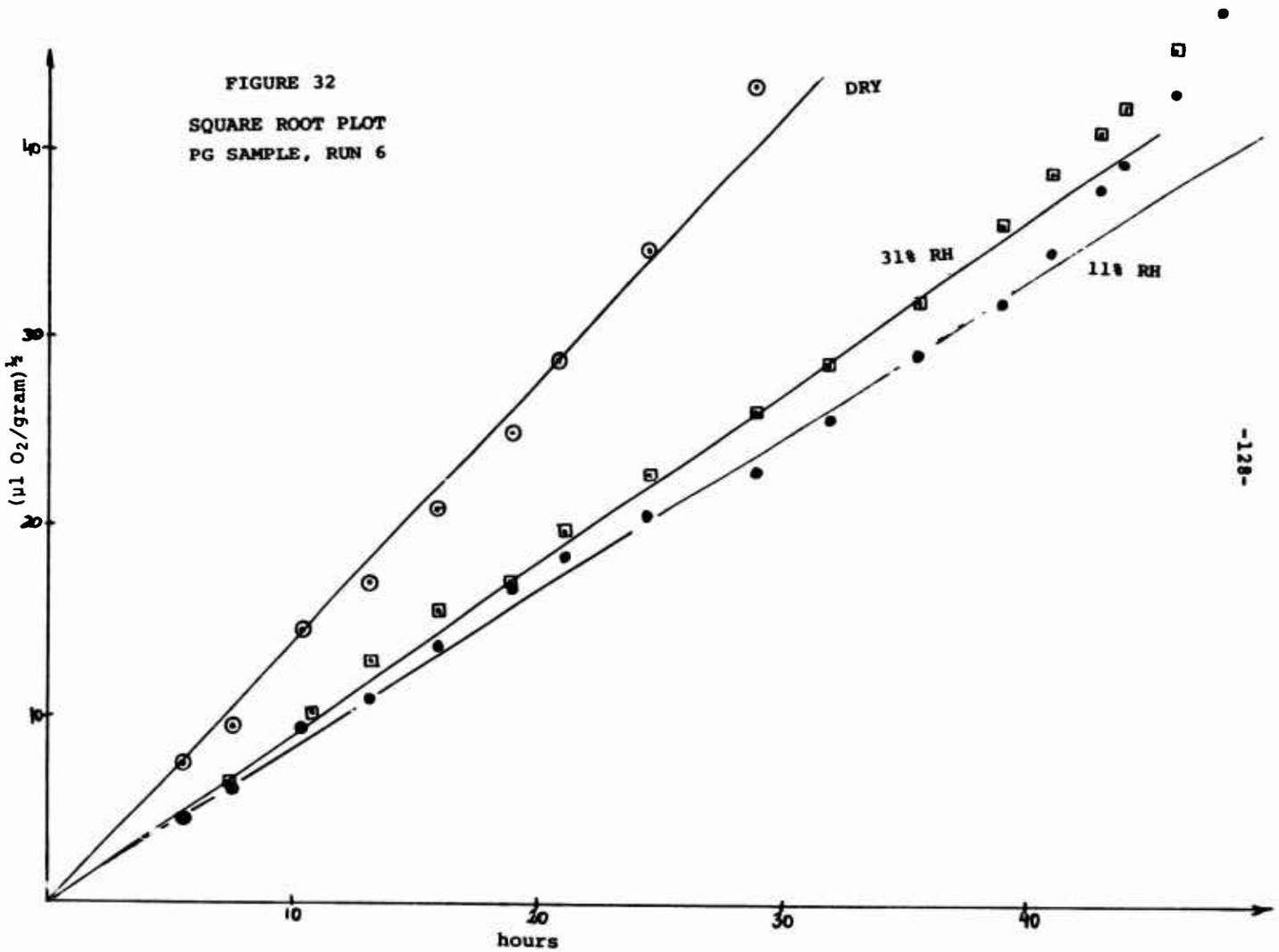
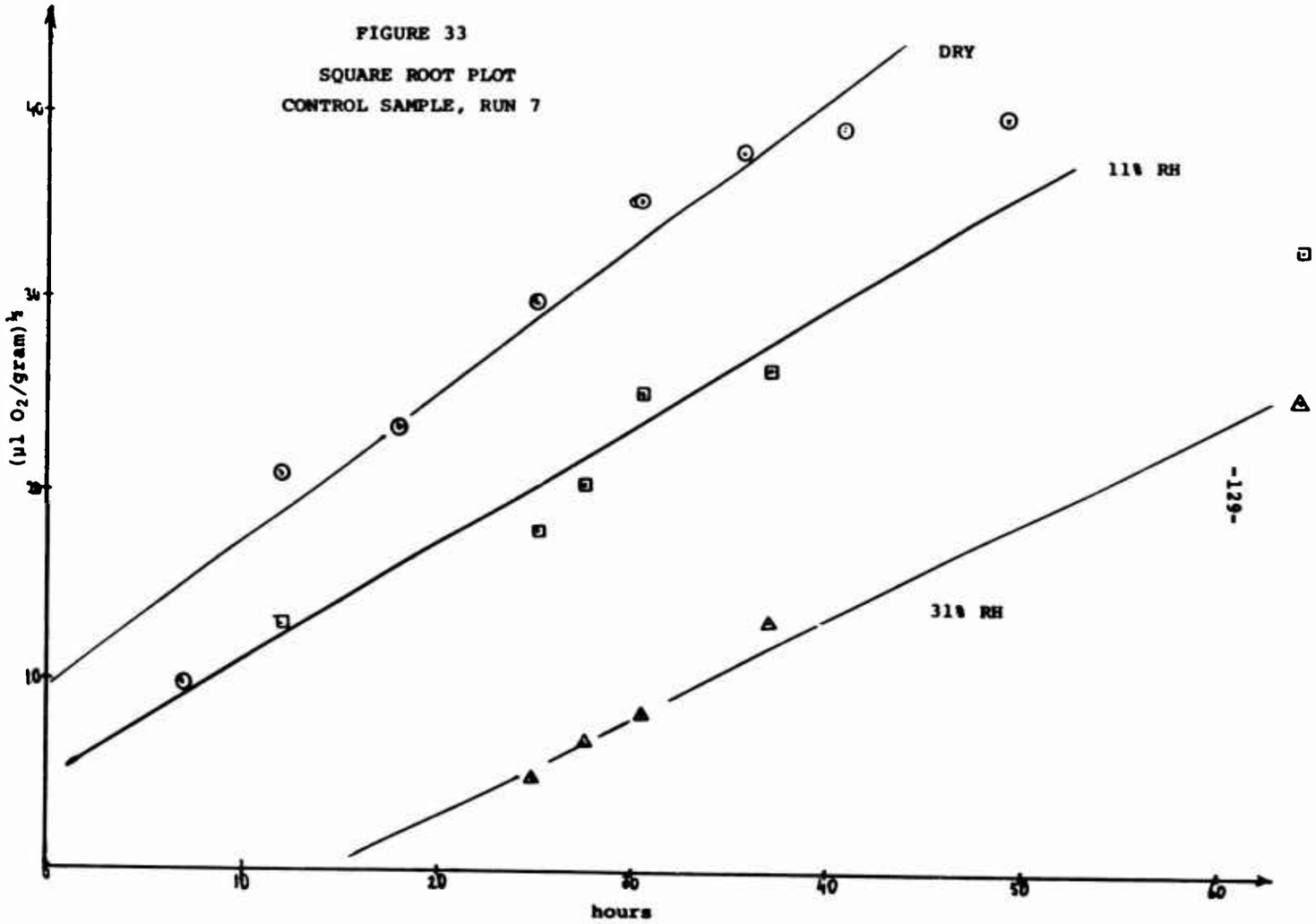


FIGURE 33

SQUARE ROOT PLOT

CONTROL SAMPLE, RUN 7



APPENDIX

A study by F. Martinez and T. P. Labuza entitled "The Rate of Deterioration of Freeze-Dried Salmon as a Function of Relative Humidity" is included as an appendix to this report.

This study was supported only to a minor extent by the funds allocated by ~~Project~~ Contract No. AF 41-609-2981 ~~66-10~~. However, the results have pertinence to the aims of this project.

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THE RATE OF DETERIORATION OF FREEZE-DRIED SALMON AS A FUNCTION OF RELATIVE HUMIDITY. F. MARTINEZ & T. P. LABUZA.

The rates of several deteriorative reactions, including lipid oxidation, astacene pigment loss, carbon dioxide production, and production of non-enzymatic browning pigments, were studied in freeze-dried salmon at 37°C and at several relative humidities. Results previously obtained in cellulosic model systems containing methyl linoleate were confirmed by data on oxygen absorption as a function of moisture content. Both the rate of the initial peroxide monomolecular decomposition and the peroxide value decreased as the water content was increased. Astacene pigment loss was reduced significantly by the higher moisture contents; non-enzymatic browning was increased. The significance of the reactivity of water at low moisture contents was demonstrated by its effect on the various reactions.

The Rate of Deterioration of Freeze-Dried Salmon as a Function of Relative Humidity

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SUMMARY—The rates of several deteriorative reactions, including lipid oxidation, astacene pigment loss, carbon dioxide production, and production of non-enzymatic browning pigments, were studied in freeze-dried salmon at 37°C and at several relative humidities. Results previously obtained in cellulosic model systems containing methyl linoleate were confirmed by data on oxygen absorption as a function of moisture content. Both the rate of the initial peroxide monomolecular decomposition and the peroxide value decreased as the water content was increased. Astacene pigment loss was reduced significantly by the higher moisture contents; non-enzymatic browning was increased. The significance of the reactivity of water at low moisture contents was demonstrated by its effect on the various reactions.

INTRODUCTION

Freeze-drying has become an important process for the preservation of foods because the quality of the rehydrated product is better than that of products prepared by conventional dehydration techniques. However, regardless of the success of the process itself, more investigation of the effect of the final moisture content on changes in the quality of the product during storage is necessary. The quality aspect involves reactions occurring under different conditions of time and storage, such as protein denaturation, changes in pigments, browning reactions, microbial growth, and development of oxidative rancidity. This last reaction, lipid oxidation, is one of the most important because of the highly porous nature of the product, making the lipid more accessible to oxygen, and because of the low moisture content which tends to promote oxidation.

Several studies have been carried out to determine the factors affecting the above reactions. The results of storage tests on food items, individually and in combinations, were consistent with the idea that the amount of water adsorbed on those systems has a very significant

effect (Lea, 1958; Notter *et al.*, 1959; Karel *et al.*, 1964). It has been postulated that the calculated monolayer value of moisture adsorption defines the region of highest stability for freeze-dried food items (Salwin, 1962).

The importance of the monolayer value for model systems undergoing lipid oxidation has been studied by Maloney *et al.* (1966) and Labuza *et al.* (1966). In their work it was found that the water exhibited 2 effects: hydration of metals which can catalyze the oxidation, thus rendering them inactive; and formation of hydrogen bonds with hydroperoxides produced to cause an extension of the monomolecular rate period.

In most studies of food materials, however, the type and amount of data taken cannot allow for an analysis of the true mechanism of the effect of water on the stability of the product. The present work was set up to determine the effect of moisture content on the rates of several deteriorative reactions in freeze-dried salmon during storage at 37°C. Oxidative rancidity, browning discoloration, and changes in pigments were studied at several different moisture contents corresponding to nearly dry, below monolayer, and above monolayer coverage

of water values. An analysis of the data was made to ascertain the true mechanism of the effect of water on the stability of the product in view of the work done in model systems.

MATERIALS AND METHODS

Preparation of samples

The freshest Pacific sockeye salmon (*Onocorhynchus nerka*) available was obtained from a wholesale dealer. It was frozen at -40°C, sliced into ¼-in. slabs, and freeze-dried for 60 hr at 60-100 μ Hg and room temperature in a Vacudyne pilot freeze-dryer. The vacuum was broken with pre-purified nitrogen. To eliminate the inherent variability of the material itself, the following treatment was adopted. 1) Only slices from the middle third of the fish body were used. 2) The skin and dark muscle were removed from the slices after freeze-drying. 3) The muscle near the abdominal cavity was also removed after freeze-drying. 4) The blood remaining in the tissue located adjacent to the backbone was thoroughly removed.

After freeze-drying the flesh was mixed thoroughly, and the necessary amounts were weighed into reaction flasks. Specific flasks and amounts were used for manometric studies, lipid extraction, peroxide value, pigment determination, and head-space analysis.

Moisture sorption isotherm. Using the desiccator method (Stitt, 1958), a sorption isotherm was prepared at 37°C. The plot of water activity versus moisture content is presented in Fig. 1. The value at which monolayer coverage of water occurs was calculated from the BET equation (Adamson, 1960). The monolayer was found to occur at a water activity of a = 0.19 and at a moisture content of 5% on a dry basis.

Moisture equilibration. The freeze-dried samples were equilibrated at relative humidities corresponding to moisture contents above and below the water monolayer value. Samples were placed into desiccators containing saturated salt solutions at < 0.1, 11, 32, and 40% RH, equilibrated under vacuum for 4 hr at 37°C, removed by breaking the vacuum with air at the proper relative humidity, and kept at 37°C for the subsequent study.

Moisture content determinations. To ensure complete equilibration, duplicate samples from each desiccator were used for moisture determination. This determination

was made by measuring the equilibrium vapor pressure in the manner indicated by Stitt (1958), using the highly sensitive manometric system of Karel *et al.* (1964).

Stability studies

Oxygen absorption. The amount of oxygen absorbed by the freeze-dried fish was followed by the standard Warburg technique, using triplicate monometers and a thermobarometer for each relative humidity sample. Standard manometric procedures were used for calibration of the equipment and calculation of results (Umbreit *et al.*, 1964). The amount of sample was selected in such a way as to maintain a 1:25 ratio of sample volume to head-space volume. Results were expressed as microliters oxygen per gram lipid.

Correction for the carbon dioxide produced in the system was made by assuming that the volume of carbon dioxide produced is equal to the absorbed oxygen not detected by the manometers. Data from head-space analysis were used to obtain a correction for the volume of carbon dioxide evolved.

Lipid extraction. Triplicate samples were extracted

with 60 cc of a chloroform-methanol (3:1) mixture, shaken for 1 hr on a rotary shaker, then filtered through a Buckner funnel. The solvent was removed on a rotary evaporator (50°C, 30 in. Hg, 1 hr) and the remaining lipid weighed. The average extraction value was used for all subsequent calculations. In each case the lipid content was approximately 20% of the dry solids' weight.

Peroxide value. Results were obtained in milliequivalents oxygen per kilogram lipid by using the AOCS method. In order to establish comparisons with oxygen absorption data, these units were converted to microliters oxygen per gram lipid by multiplying by 11.2 (the conversion factor).

Head-space analysis. Head-space analysis was used as an indication of oxygen absorbed and carbon dioxide evolved. Two grams of fish were put into 50-cc Erlenmeyer flasks and stoppered with a rubber serum cap. Using the procedure described by Karel *et al.* (1963), 1 cc of the head space was injected during storage into an Aerograph Model A-90 P Gas Chromatograph with a thermal conductivity cell.

Peak heights were compared with standards of known

gas concentration, the results being expressed as percent of the head space.

Production of browning pigments. Pigments were measured according to the method presented below (modified from Choi *et al.*, 1949).

Two grams of dry material were dispersed in distilled water (20 cc). A trypsin suspension of 2.5 ml, prepared a few minutes before the analysis, was added. After a 1-hr incubation at 45°C, 2 ml of 50% trichloroacetic acid and 0.1 g Celite (Analytical filter aid) were added. After mixing and filtering, the optical density of the filtrate was measured at 420 mμ, setting the enzyme blank at 100% T. The readings were made in a Beckman B Spectrophotometer.

Astacene pigment loss. The pigment was extracted by the method developed by Bligh *et al.* (1959). The optical density of the chloroform extract of the pigment was measured at 475 mμ in a Beckman B Spectrophotometer. A reading at 390 mμ was used as a reference point to eliminate differences in the absolute amount of pigment extracted (Lusk *et al.*, 1964). The data are reported as the ratio of the optical densities at 475 and 390 mμ (OD 475/OD 390).

Fatty acid analysis

In order to make comparisons with runs in which a model system containing methyl linoleate was used, and to study certain aspects of reaction kinetics on the basis of methyl linoleate alone, the fatty acid composition of the freeze-dried material was determined.

Triplicate samples were methylated according to the trans-esterification procedure (no. 1) (Jamieson *et al.*, 1965). The methyl esters were dissolved in hexane and then examined on a F & M Model 1609 gas chromatograph.

Comparison of the retention times of the fatty acid methyl esters obtained from the fish lipids with the retention times of fatty acid methyl esters of known concentration obtained from the Hormel Institute and from Applied Science Laboratories, Inc. led to identification. Conventional procedures were used for peak area measurements.

RESULTS AND DISCUSSION

Three experiments varying from 250-900 hr were carried out on the freeze-dried salmon. As many treatments as possible were considered in each test because of the inherent variability of the fish (Lovern,

1942; Notevarp, 1961). Although the absolute magnitudes of values varied between the runs in each case, the trend observed was the same. Results are presented from typical values observed.

Oxidative deterioration

Oxidative deterioration was evaluated by measurement of the oxidation of the lipids, the protein fraction, and the destruction of astacene, the pigment responsible for the typical red color of fresh salmon. Figs. 2 and 3 show the data, corrected for the amount of carbon dioxide produced by the sample, for the Warburg oxygen absorption of salmon. It can be seen that the major effect of humidification is to significantly decrease the over-all rate of oxidation after an initial fast phase. The protective effect of water is evident both below the monolayer (11% RH) and above it in all cases. These results are similar to that found by Maloney *et al.* (1966) for cellulosic model systems.

As was observed by Tappel (1956) and by Karel *et al.* (1966), oxygen can also be absorbed by proteins. This can account for up to 50-80% of the total oxygen absorbed, especially at high temperatures. In order to

test for such absorption in the salmon system, in each run samples which had been extracted 6 times with $CHCl_3$:MeOH (3:1) were also placed in Warburg manometers. Very little absorption was observed; in all cases it was less than 1% of that found for the unextracted fish, so that protein oxidation was assumed to be negligible.

Table 1 presents data obtained by the use of head-space analysis of salmon samples and converted to microliters O_2 absorbed per gram of lipid in the fish. A comparison of this data with the Warburg data in Fig. 3 (for run 3) shows some discrepancy, although the general trend of lower values at the higher moisture content was evidenced. These discrepancies could be due to inhomogeneity of the sample since a different sample was used for each point. Similar difficulties have been reported by Vilece *et al.* (1955) and by Karel *et al.* (1966).

The results of peroxide value analyses are presented in Fig. 4. The peroxide values varied inversely with the amount of water present in the system, showing the protective effect of water.

Comparison of the peroxide value, in microliters of oxygen per gram of lipid, with the Warburg oxygen absorption data shows that the peroxide value gives a very poor

indication of the actual total amount of oxygen absorbed by stored foods. This is the opposite of results found in a cellulosic model system containing methyl linoleate. This model system was prepared in the manner described by Maloney *et al.* (1966). Oxygen absorption and peroxide value analyses were performed by following the methods described in the present work; the results are plotted in Fig. 5 and show that the correlation between peroxide value and oxygen absorbed is very good. Lundberg *et al.* (1947) studied the bulk oxidation of methyl linoleate at various temperatures and found a direct correlation between these parameters at temperatures near 40°C. The reasons for the poor correlation of peroxide value with oxygen absorbed in foods could be due to rapid breakdown of the hydroperoxides due to secondary reactions, significant oxygen absorption by components other than lipid (Tappel, 1956), or irreversible chemical bonding of the peroxides to other components such as proteins, as found by Tappel (1955) and by El-Gharbawi *et al.* (1965).

It can be seen from Fig. 4 that even though the peroxide value does not show the total quantitative oxygen absorbed, the protective effect of water is readily observed if enough determinations are made

over the extent of the storage period.

The effect of moisture content on the oxidative deterioration of astacene is presented in Fig. 6 for run 3. The effect is similar to that found for lipid oxidation, with values above the monolayer giving almost complete protection. Lusk *et al.* (1964) discussed the sensitivity of the pigment when exposed even to low oxygen partial pressure, with stability obtained only in pure nitrogen at 0°F. The present results show that complete stability can be obtained at 37°C in air by choosing the proper moisture content.

Non-enzymatic browning

The extent of non-enzymatic browning deterioration was evaluated by determining the relative amounts of pigments and carbon dioxide produced during the course of the reaction. Because the data was obtained from analyses performed on different samples and homogeneity may not have been attained during their preparation, the variability of the results is large.

Production of brown pigments. The results of determinations performed on aqueous extracts of similar quantities of freeze-dried material are presented as

OD $\times$ 100 versus time in Figs. 7 and 8 for 2-g samples. It can be seen that the rate of production of pigments was a function of the amount of water in the material studied, except in the case of the dry sample (run 2, Fig. 7) where a secondary reaction presumably complicated the mechanism. It is obvious that 2 types of browning took place: one in which the residual free reducing sugars present in the freeze-dehydrated product participated in reactions with the amino groups of the protein and one in which the decomposition products of oxidized lipid probably reacted with the amino groups of the protein.

According to Jones (1962) glucose, ribose, and phosphorylated hexoses are the most important compounds responsible for sugar-amino reactions in dried fish products. The significance of the relative amounts of free reducing sugars varies, depending upon many factors including physiological conditions and processing variables.

The curves obtained for the samples equilibrated at 11, 32, and 40% RH are characteristic of a non-enzymatic browning process taking place in a system where the concentration of reducing sugars

has become limiting after a certain reaction time. Also, it is clear that the rates are proportional to the amount of water available for the transport of the reactants in solution. Since the rate of oxidation of the samples was very slow at this time, the observed browning probably occurs via an interaction of free reducing sugars and amino groups of the protein.

The sudden increase in the rate of browning exhibited by the dry samples (Fig. 7) can be explained on the basis of the reaction of protein end groups with products of oxidation. After 200 hr of storage, pigments were produced at a very high rate in the sample being oxidized. This indicated that the decomposition products of oxidized lipids, probably aldehydes and ketones, may be responsible for the initiation of non-enzymatic browning reactions at levels of moisture near dryness.

Since samples equilibrated at 11 and 32% RH oxidized very slowly, the concentration of lipid degradation products present throughout the storage period should also be very small. Therefore, at these levels of moisture content the browning reaction was carried out primarily by a sugar-amino mechanism. As was also found in the work reported here, Tarr *et al.* (1965) concluded

that browning at moisture contents near dryness was due mainly to the interaction of very reactive sugars and products of lipid oxidative reactions with the amino groups of the protein. Results of GLC head-space determinations are presented as percent carbon dioxide in head space versus time in Fig. 9. The data represented in this figure show that the evolution of carbon dioxide paralleled the production of pigments, indicating that carbon dioxide is produced during browning and is probably produced through Strecker degradation.

Kinetics of deterioration

Effect of humidification on the rate of lipid oxidation of freeze-dried salmon. Examination of the oxygen absorption curves in Figs. 2 and 3 indicates that one of the effects of water is a reduction of the relative amounts of oxygen absorbed. To elucidate the actual mechanism for this protective effect, the results were recalculated on the basis of oxidizable lipid. It has been demonstrated (Lundberg, 1962) that the rate of oxidation of fatty acids containing less than 18 carbon atoms and less than 2 double bonds is negligible compared

to that of linoleic acid.

In Table 2 the results of the fatty acid analysis performed on freeze-dried salmon are compared with the results obtained by other investigators. It can be seen that the amount of linolenic acid is negligible, and, in consequence, it is reasonable to assume that linoleic acid is the main fatty acid responsible for the observed oxygen uptake. The other fatty acids found were either non-oxidizable or were found only in trace amounts.

If every oxygen molecule reacts with 1 molecule of linoleic acid to yield a linoleic acid hydroperoxide, and if the concentration of linoleic acid is large enough to be considered constant throughout the oxidative period, then the plot of the square root of the oxygen absorbed per gram of linoleic acid versus time will be a straight line whose slope is a measure of the monomolecular rate constant (Maloney *et al.*, 1966). A monomolecular rate plot for run 2 is presented in Fig. 10.

With the model system previously discussed, the reaction proceeds by a monomolecular decomposition of the hydroperoxides until a value corresponding to approximately $25(\mu l\ O_2$ absorbed per gram lipid$)^{\frac{1}{2}}$ is reached; the change in slope obtained afterwards indicates

that the bimolecular phase of oxidation is taking place with the breakpoint increasing with moisture conten. (Karel *et al.*, 1967). The data obtained for the salmon demonstrates the following 3 points.

Since no upward breakpoint exists, the oxidation of the lipids of freeze-dried salmon does not enter into the rapid bimolecular decomposition phase. This result is reasonable since in complex materials, such as foods, peroxides formed have less opportunity to reach each other and to decompose bimolecularly.

The data do not fit into a straight line very well. The reason for this is that the amount of oxidizable lipid (linoleic) may not be constant throughout the observation period; however, it could become limiting after a certain reaction time, thus causing the monomolecular rate plot to curve downward. The results are based on a lipid composition taken from a single fish; because of reasons mentioned previously, the true value may vary considerably.

Since some browning occurred with increased moisture content in the salmon, end products of browning may be responsible for the decrease in rate through an anti-oxidant mechanism.

Monomolecular rate constants obtained for the salmon and for the model system are compared in Table 3, which shows that the effect of moisture was exerted by a lowering of the monomolecular rate constants. This indicates the possible occurrence of hydrogen bonding of hydroperoxides by water, thereby preventing their entering into initiation reactions, and of inactivation of metal catalysts by water. These results are in agreement with the work of Maloney *et al.* (1966) and Labuza *et al.* (1966).

Effect of humidification on the rate of astacene deterioration. According to Lusk *et al.* (1964) the pigment astacene is extremely sensitive to oxidation even at very low oxygen pressures; therefore, if the concentration of oxygen is not limiting, a first-order mechanism can be proposed with the rate of the reaction at any instant proportional to the concentration of substrate left. Thus,

$$\ln\frac{A}{Ao} = -K\,\Theta$$

where Ao is the initial amount of pigment;

A is the amount present at time, Θ;

K is the first-order constant for the reaction; and

Θ is time.

In consequence, a plot of $\ln\frac{A}{Ao}$ versus θ will be a straight line whose slope is a measure of K. This same analysis has been applied by Falconer *et al.* (1964) in a study of the oxidation of carotene in dehydrated carrot.

The first-order rate plot for the salmon is presented in Fig. 11, and the numerical values for the constants obtained as a function of different humidification treatments are presented in Table 4. From this data it is possible to conclude the following.

Water exerted a marked effect by lowering the rate of deterioration of astacene.

The protective effect of moisture was manifested continuously from the multilayer region to the dry state. The actual manner by which water protects astacene from oxidation cannot be truly elucidated. Since the data nearly fits into a straight line, demonstrating that the concentration of oxygen is not limiting, the theory proposed by Halton *et al.* (1937) and by Salwin (1962), in the sense that water forms a protective film excluding oxygen from the surface, cannot be of significance. However, there are 2 mechanisms which can be operative in the system studied. The oxidative reaction is probably

affected by metal catalysis, and water hydrates metals, thus rendering them inactive. Secondly, although it followed this type of plot over the range studied, the reaction may not be a real first-order one. If the reaction obeys a free radical mechanism, then, as in the case of lipids, the effect of water on the oxidative deterioration would be exerted.

Effect of humidification on the rate of non-enzymatic browning. According to Ellis (1959) the amount of pigment formed during non-enzymatic browning reactions is directly proportional to the square of time elapsed and to the concentration of reactants. Thus,

$$\text{browning pigment} = K(A)^2(S)\theta^2$$

where A is the concentration of amino acid end groups;

S is the concentration of reducing sugars;

θ is the reaction time; and

K is the rate constant.

In the salmon system the amount of amino acid end groups is large and, secondly, the carbonyl compounds produced during the oxidation of lipids can accomplish the role of sugars (in addition to the free sugars present). Therefore, the above equation can be reduced to

$$OD_{420} = K'(Y)\theta^2$$

where OD_{420} is the concentration of pigment formed, and

Y is the total quantity of reducing substances capable of undergoing the reaction.

A plot of the concentration of pigment formed (OD_{420}) versus the square of the time elapsed will be a straight line whose slope is proportional to the rate of the reaction and to the concentration of reducing compounds available. This plot is presented in Fig. 12 for run 3 and in Fig. 13 for run 2. A sudden increase in the slope is attained during the period when the oxidation of the more reactive lipids probably takes place. This fact supports the observation made in the present study by showing that products of the autoxidation reaction of lipids probably interact with the amino groups of the protein to form carbon dioxide and browning pigments. In addition, at the end of the period where the rate of lipid oxidation was rapid the sudden rise in browning pigments stopped. This could be explained by the dual role of oxidation and browning as a function of moisture content. The water promoted browning which produced anti-oxidants, thus causing the rapid oxidation period to cease.

CONCLUSIONS

In the storage of dehydrated food the equilibrium relative humidity of the final product will largely determine the storage life of a food. In a product such as salmon a balance must be achieved among the various deteriorative reactions in order to predict the maximum storage life. Since it has been shown that, even below the monolayer, water exerts a distinct effect, the use of the monolayer value as the optimum condition may not be desirable. In this study it was found that at 32% RH the salmon showed the best over-all stability. This is in excess of the monolayer and far in excess of the recommended 2% moisture content maximum for most dehydrated foods.

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Table 1. Head-space oxygen absorption: Run 3.

(μl O_2/g lipid)

Time (hr)	Dry < 0.1% RH	Below monolayer 11% RH	Above monolayer 32% RH	Above monolayer 40% RH
180	2,381	2,509	2,509	1,314
305	3,423	3,711	3,134	1,346
425	4,363	4,641	4,208	1,570
595	4,064	4,192	3,775	2,564
765	4,272	4,817	3,711	2,067

Table 2. Fatty acid composition of freeze-dried salmon.

Fatty acid	Total lipid (% by wt)	Saddler *et al.* (1966)	USDA (1963)
Lauric	trace		
Myristic	0.52		
Palmitic	2.6		
Stearic	0.61		
Oleic	0.26		
Linoleic	4.8	11.7	1.4
Linolenic	0.17	1.0	—
Arachidic	0.60		

Table 3. Monomolecular rate constants for freeze-dried salmon. ([μl O_2/g linoleate]$^{\frac{1}{2}}$ per hr)

Samples	Dry < 0.1% RH	Below monolayer ~ 11% RH	Above monolayer ~ 32% RH	Above monolayer ~ 40% RH
Model system[a]	0.47	0.30	—	—
	0.94	—	0.66	—
	1.23	—	—	1.0
Salmon: Run 2	0.54	0.38	0.24	—
Run 3	0.5	0.5	0.43	0.36

[a] Data taken from Karel *et al.*, 1967.

Table 4. Astacene pigment rate constant.

(hr^{-1})

	Dry	Below monolayer	Above monolayer	
	< 0.1% RH	11% RH	32% RH	40% RH
Run 2	1.1×10^{-3}	0.9×10^{-3}	0.6×10^{-3}	—
Run 3	1.1×10^{-3}	1.0×10^{-3}	0.5×10^{-3}	0.1×10^{-3}

FIGURE 1

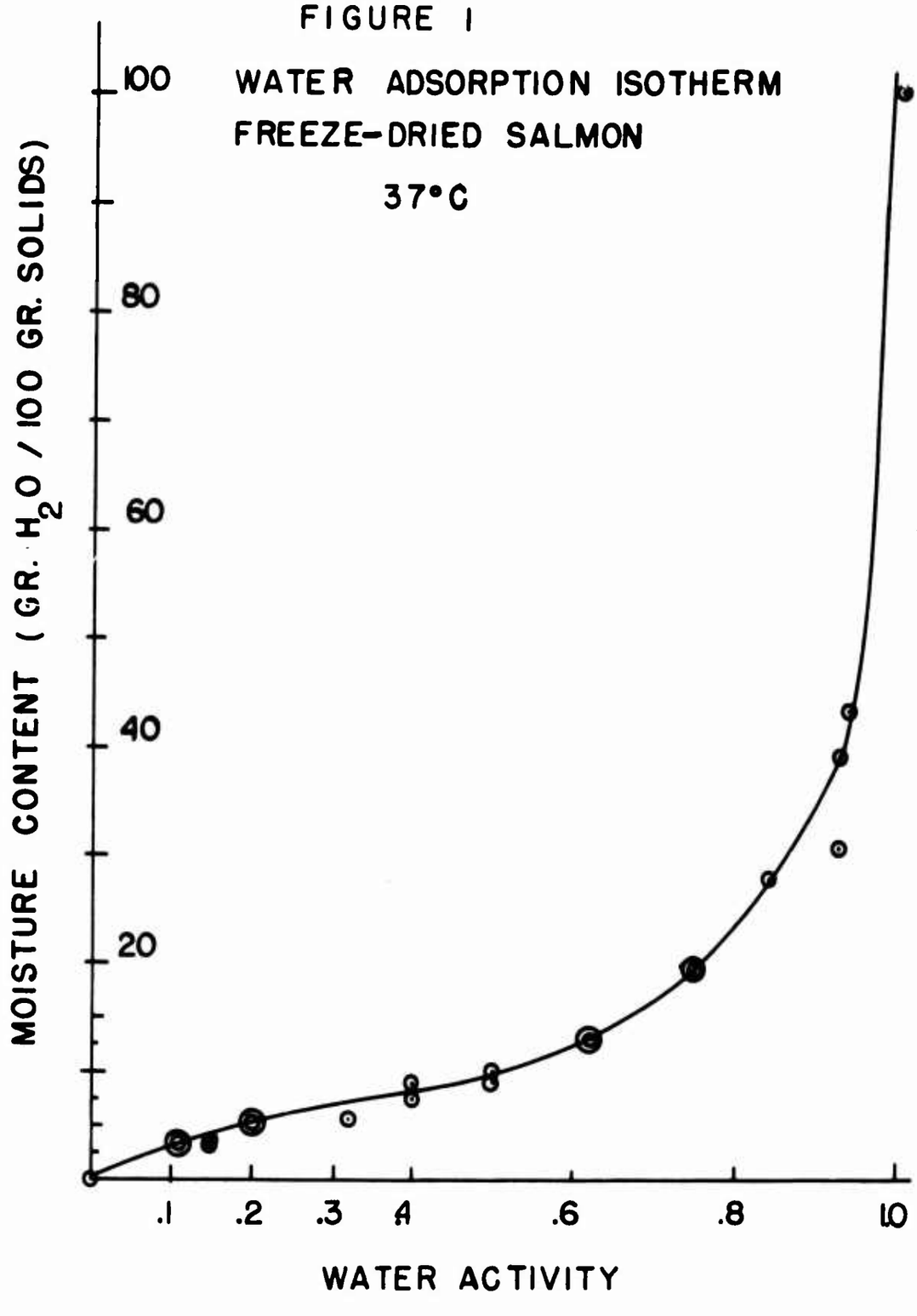

WATER ADSORPTION ISOTHERM
FREEZE-DRIED SALMON
37°C
MOISTURE CONTENT (GR. H_2O / 100 GR. SOLIDS)
100
80
60
40
20
.1
.2
.3
.4
.6
.8
1.0
WATER ACTIVITY

FIGURE 2
FREEZE-DRIED SALMON - RUN N° 2
CORRECTED OXYGEN ABSORPTION

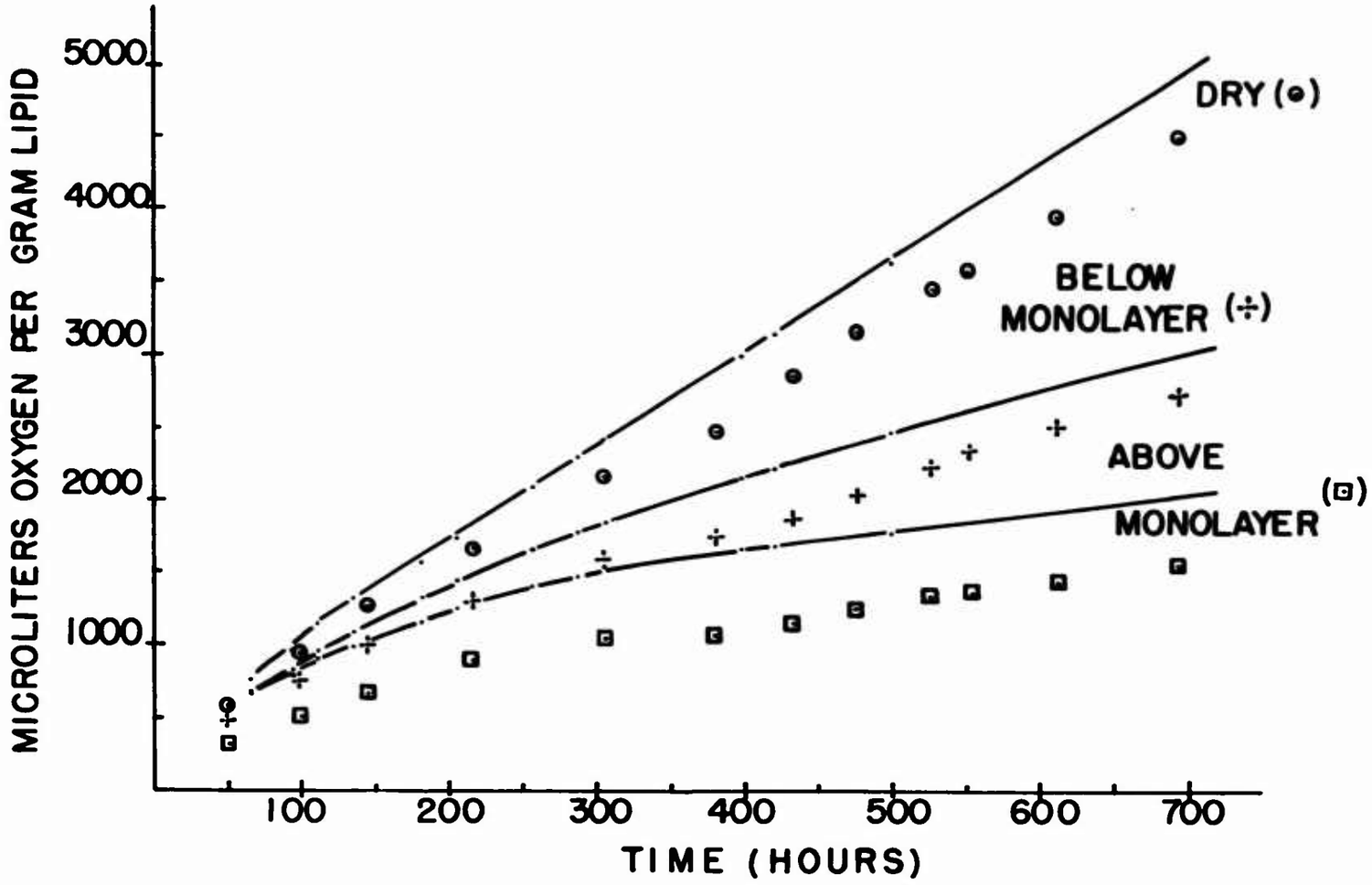


FIGURE 3
FREEZE-DRIED SALMON - RUN N° 3
CORRECTED OXYGEN ABSORPTION

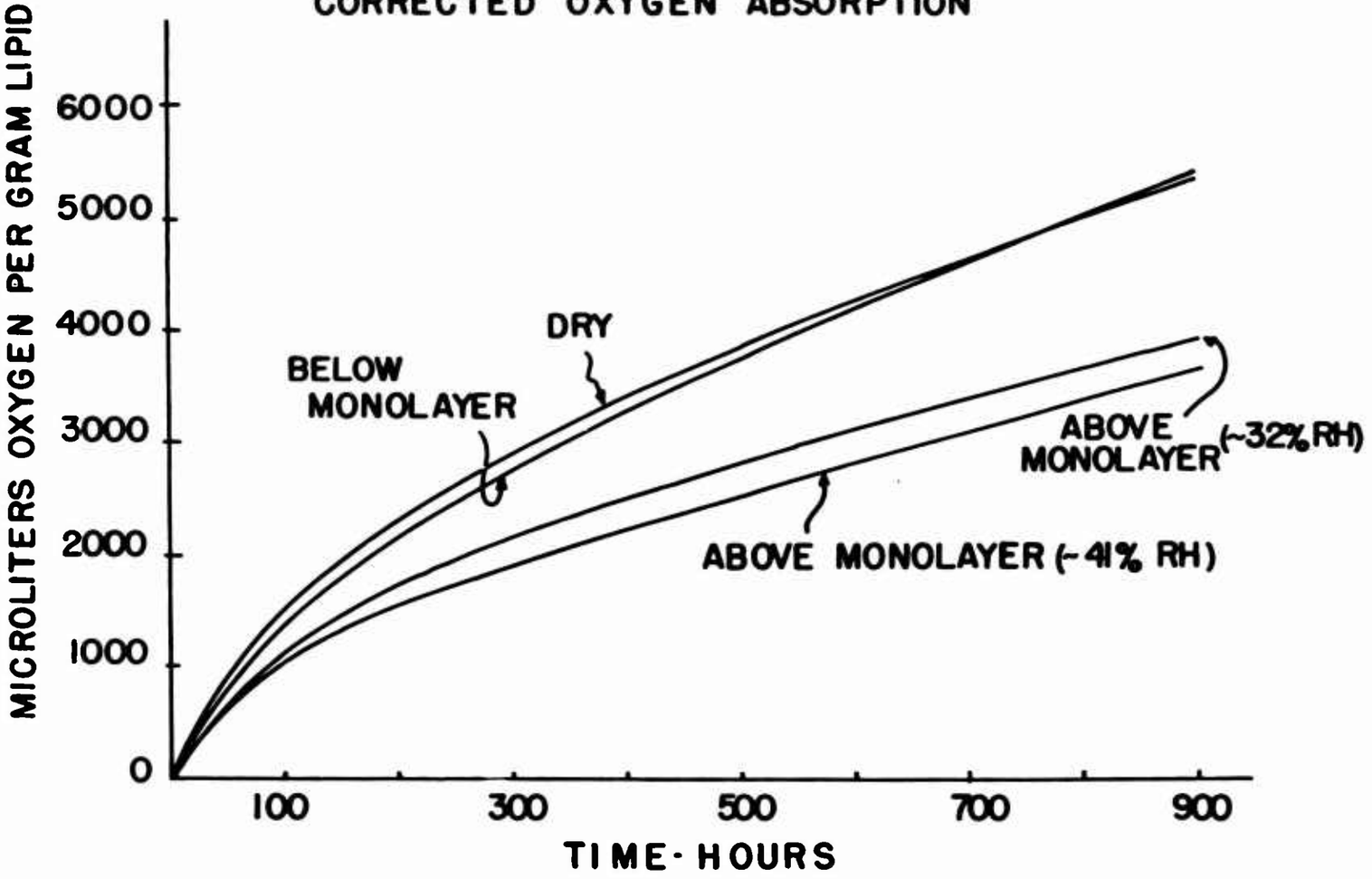


FIGURE 4

FREEZE-DRIED SALMON-RUN N° 3

PEROXIDE VALUE

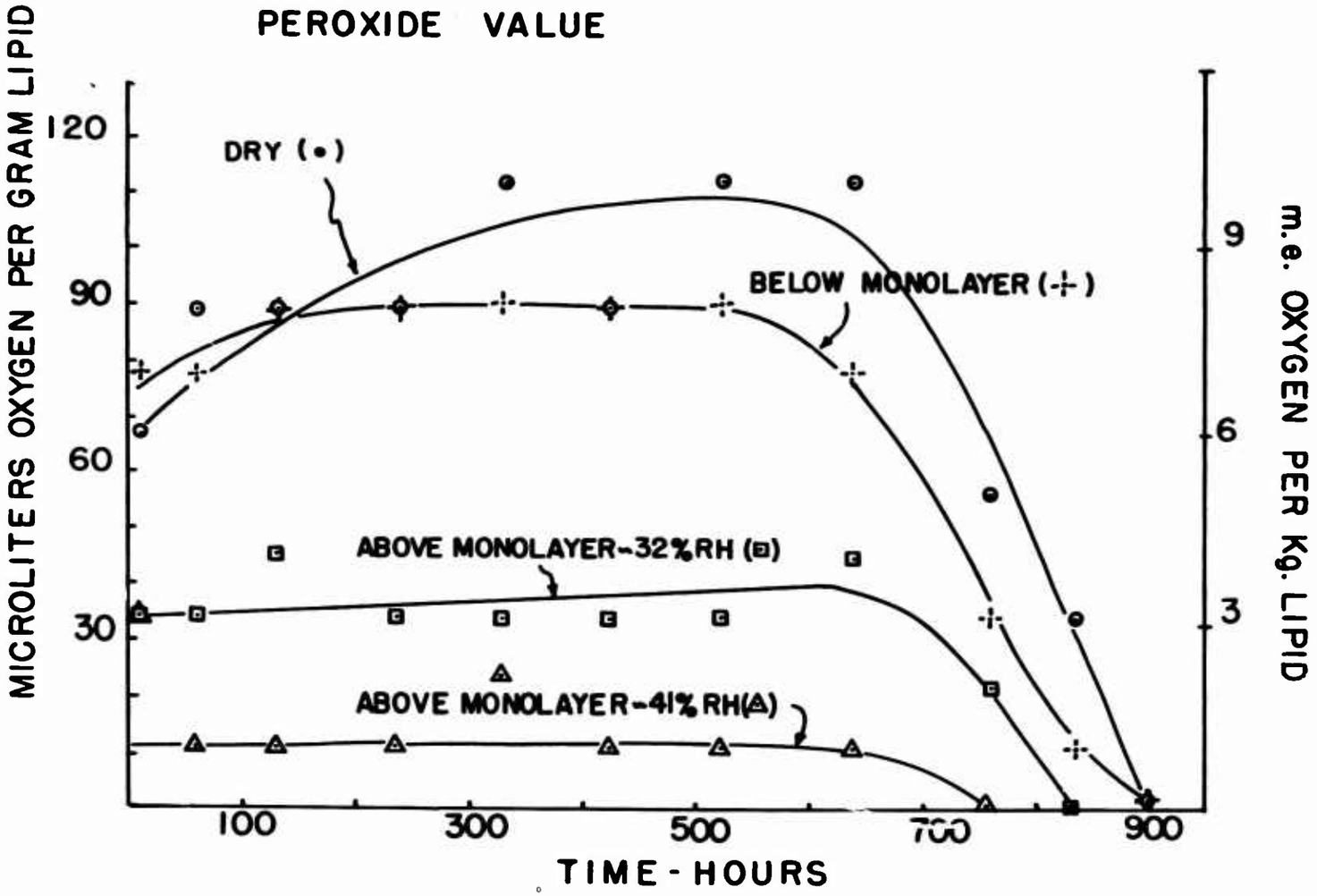


FIGURE 5

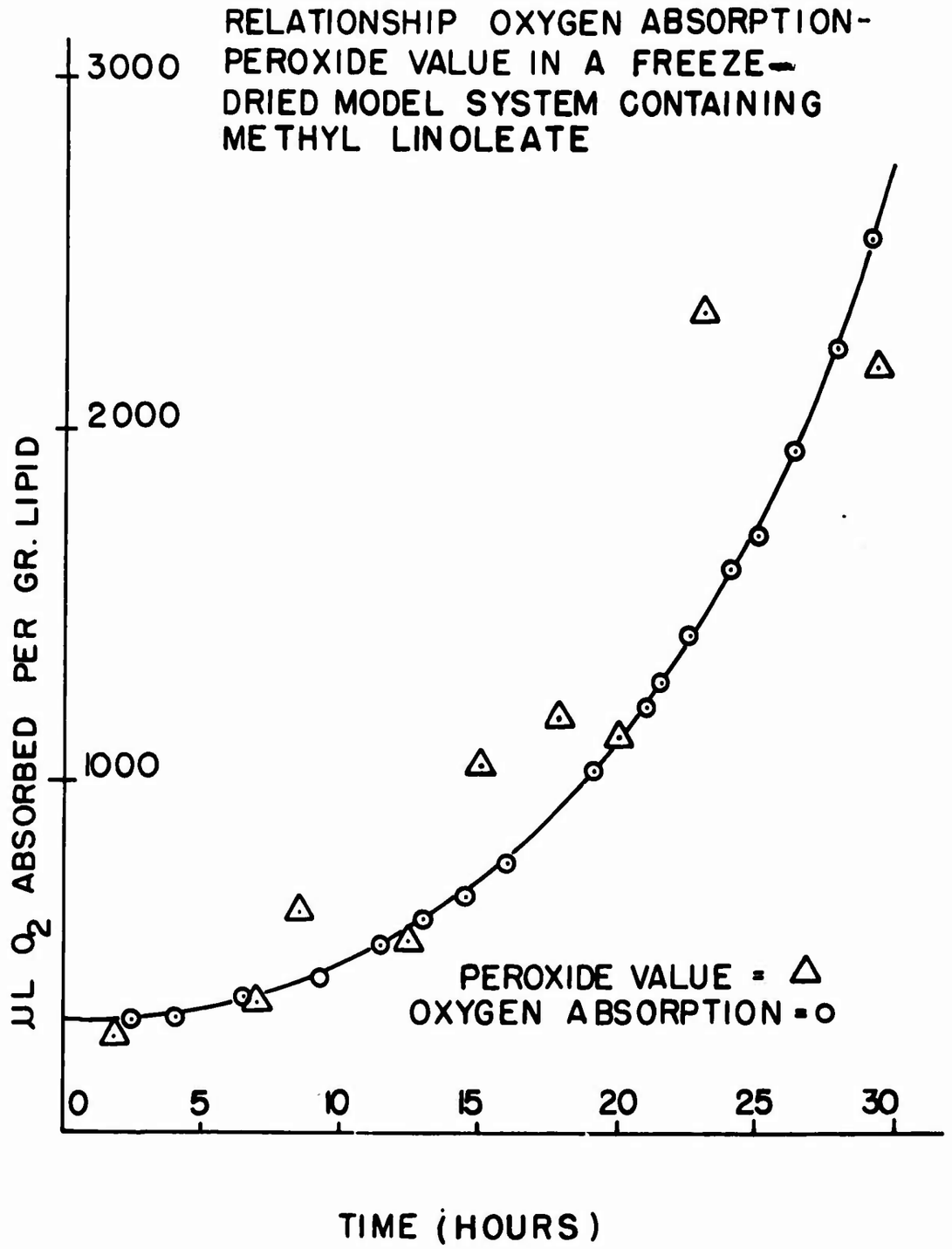

RELATIONSHIP OXYGEN ABSORPTION-
PEROXIDE VALUE IN A FREEZE-
DRIED MODEL SYSTEM CONTAINING
METHYL LINOLEATE
3000
2000
1000
0
5
10
15
20
25
30
µL O2 ABSORBED PER GR. LIPID
PEROXIDE VALUE = △
OXYGEN ABSORPTION = o
TIME (HOURS)

FIGURE 6

FREEZE-DRIED SALMON-RUN N°3

ASTACENE PIGMENT DETERIORATION

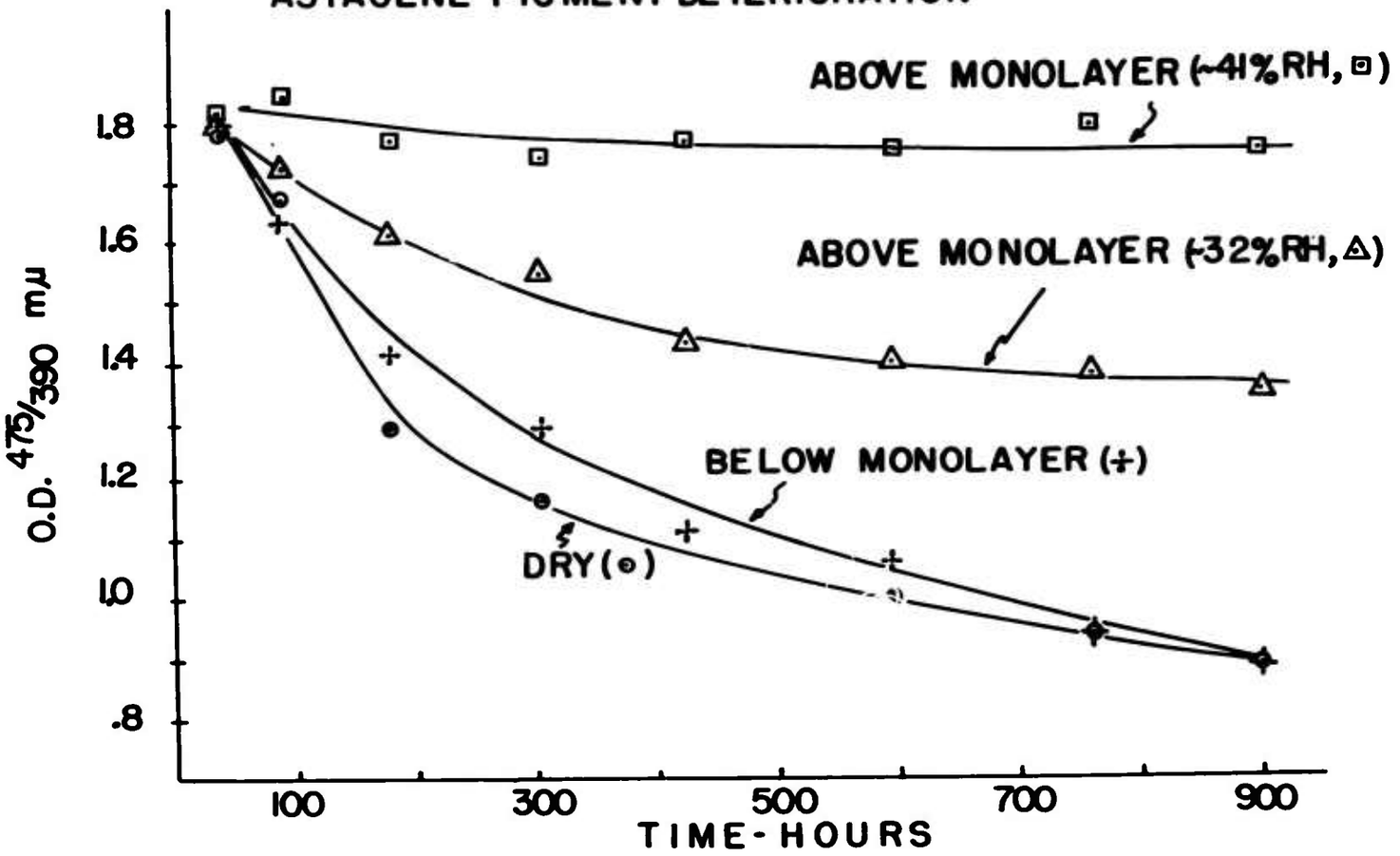


FIGURE 7

FREEZE-DRIED SALMON- RUN N° 2

BROWNING PIGMENTS PRODUCTION

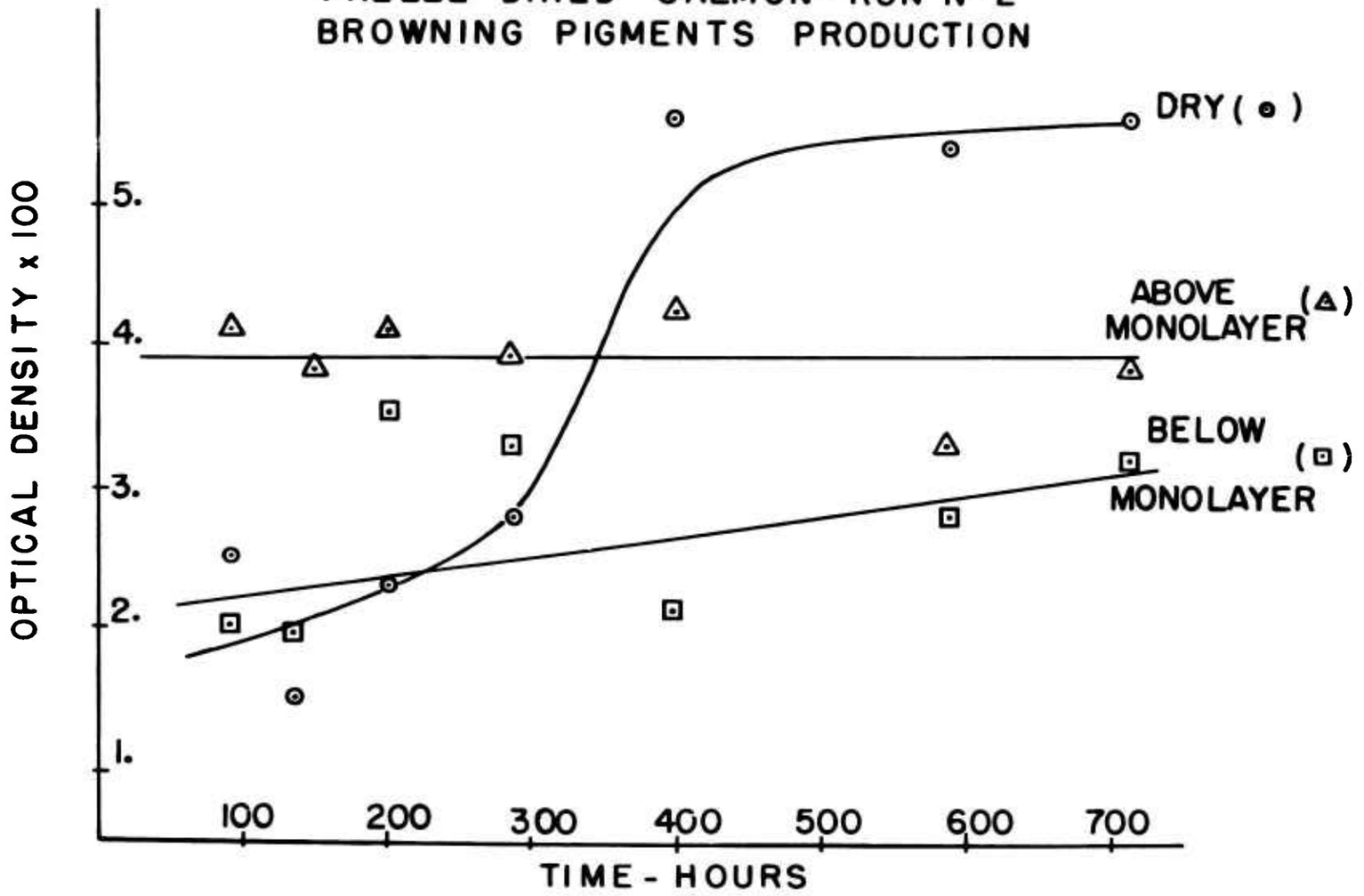


FIGURE 8

FREEZE-DRIED SALMON-RUN N°3
BROWNING PIGMENTS PRODUCTION

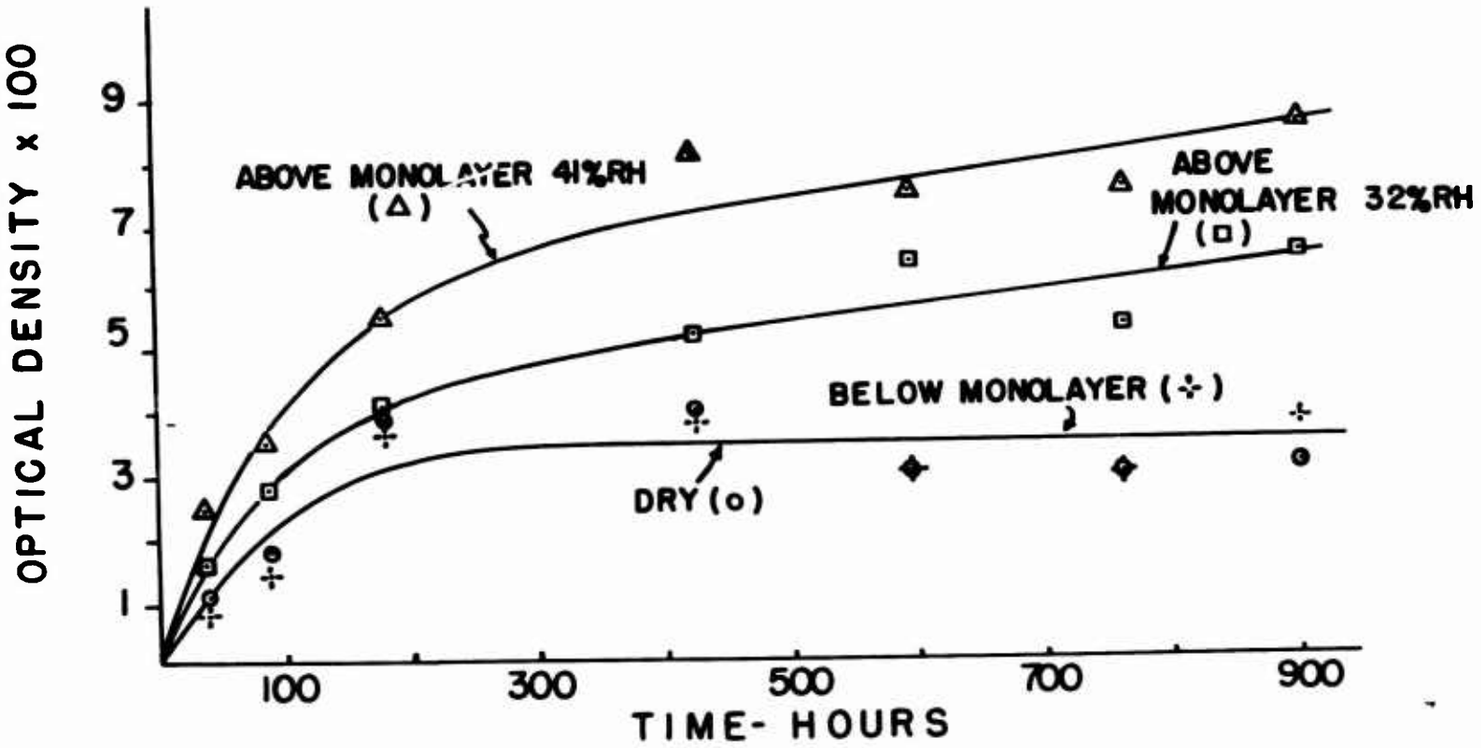


FIGURE 9

FREEZE-DRIED SALMON - RUN N°3

CARBON DIOXIDE PRODUCTION

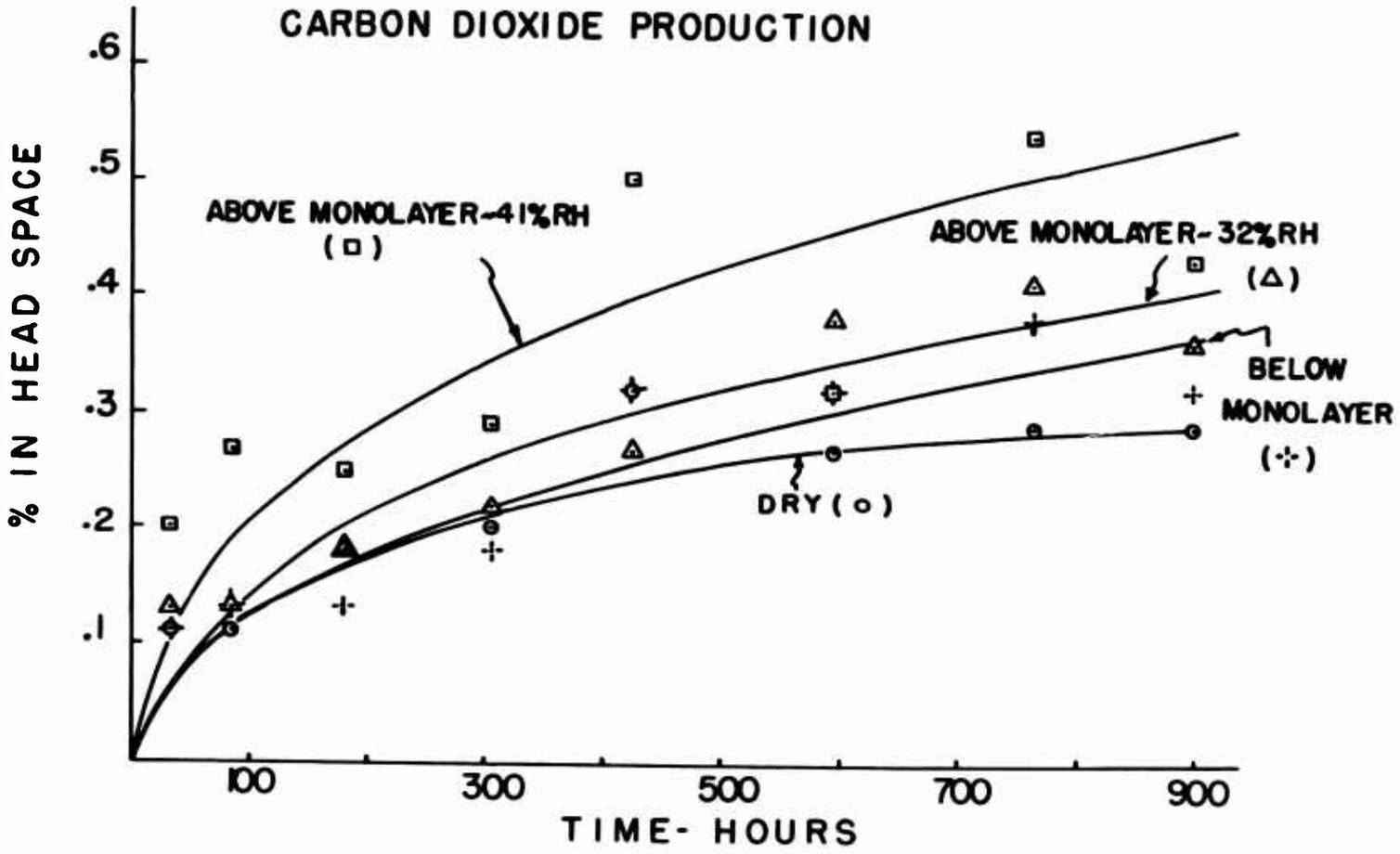


FIGURE 10

FREEZE-DRIED SALMON - RUN Nº 2

MONOMOLECULAR RATE PLOT

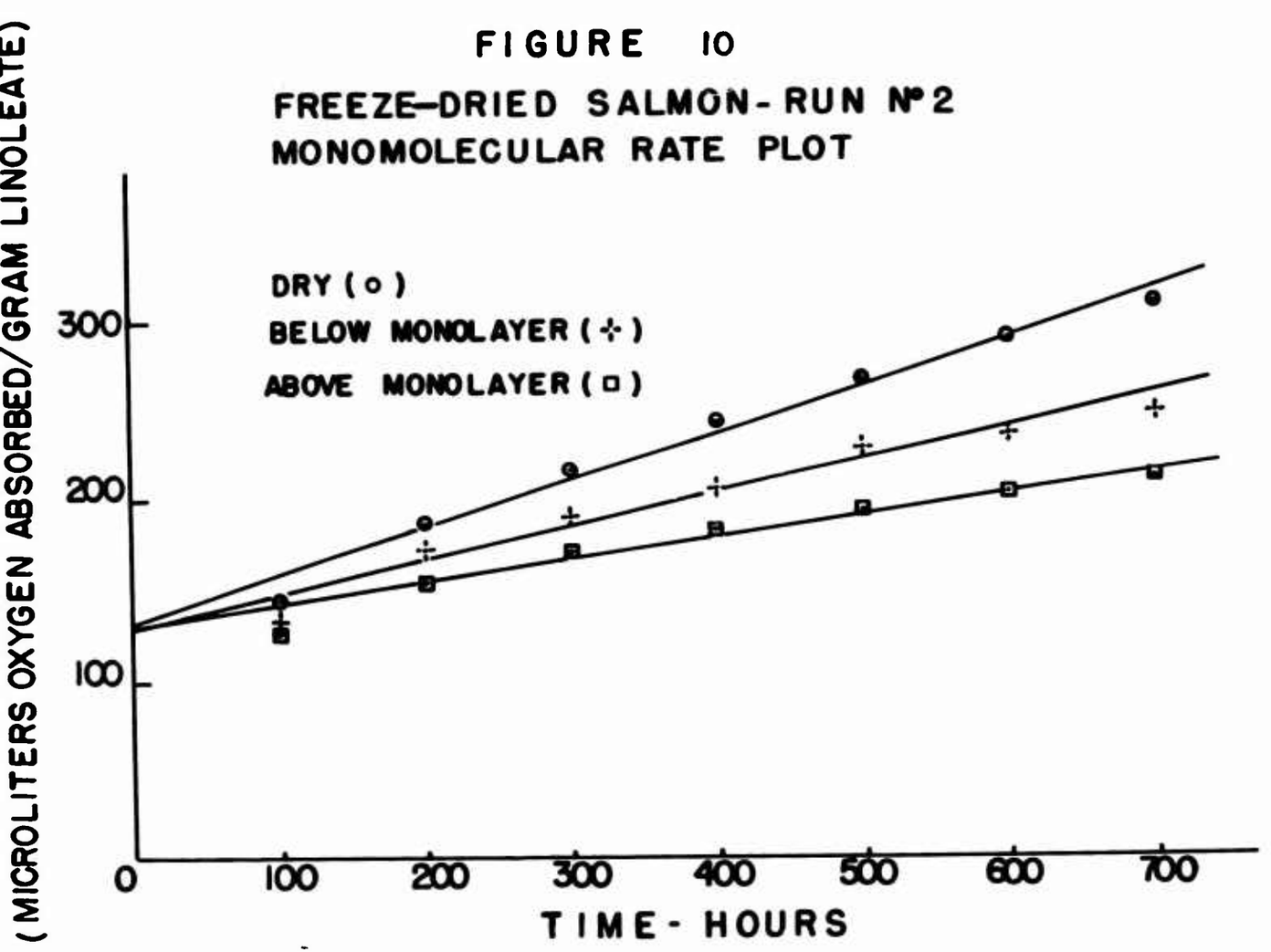


FIGURE II

FREEZE-DRIED SALMON - RUN N° 2

ASTACENE DETERIORATION - FIRST-ORDER PLOT

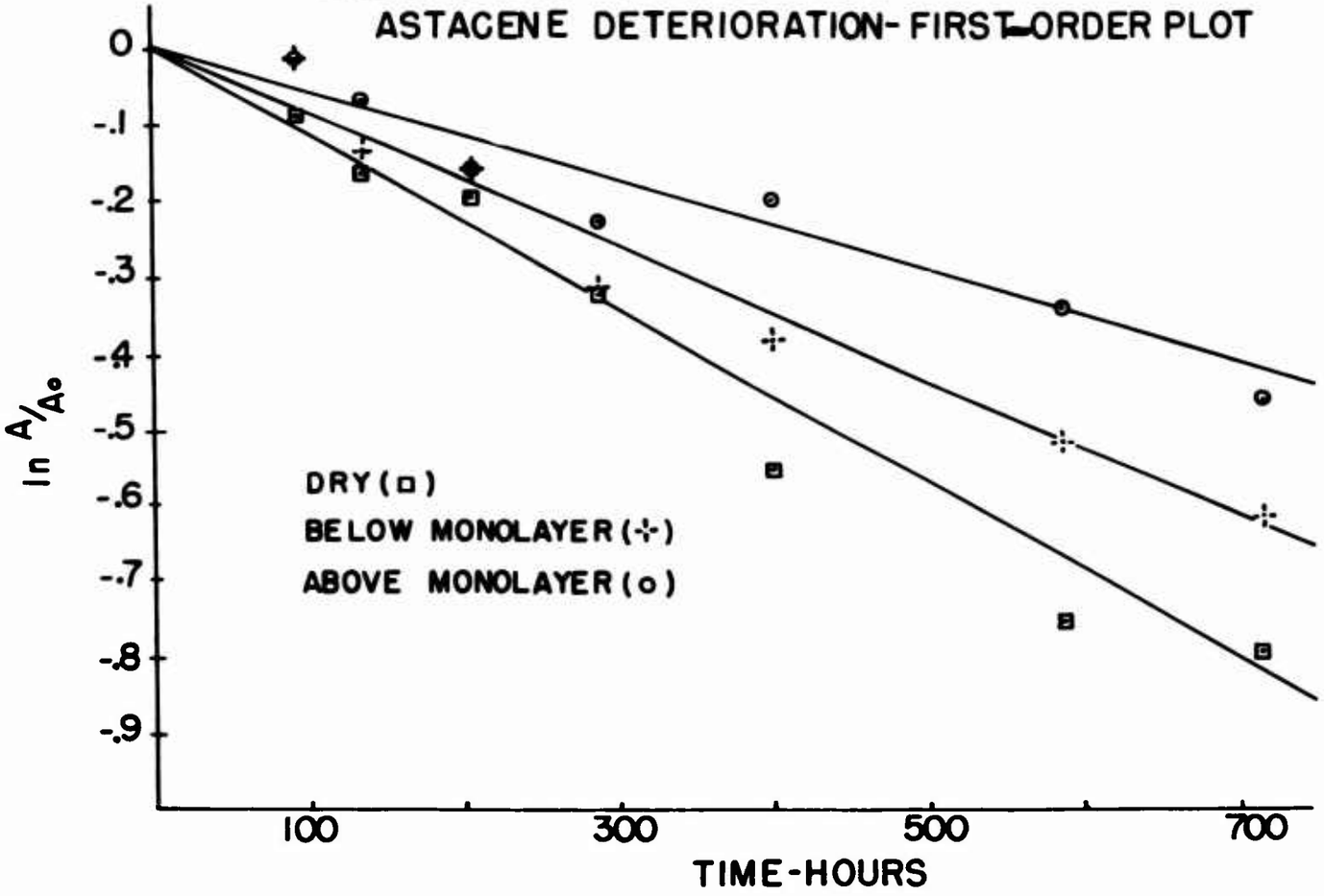


FIGURE 12

FREEZE-DRIED SALMON - RUN N°3
BROWNING KINETICS

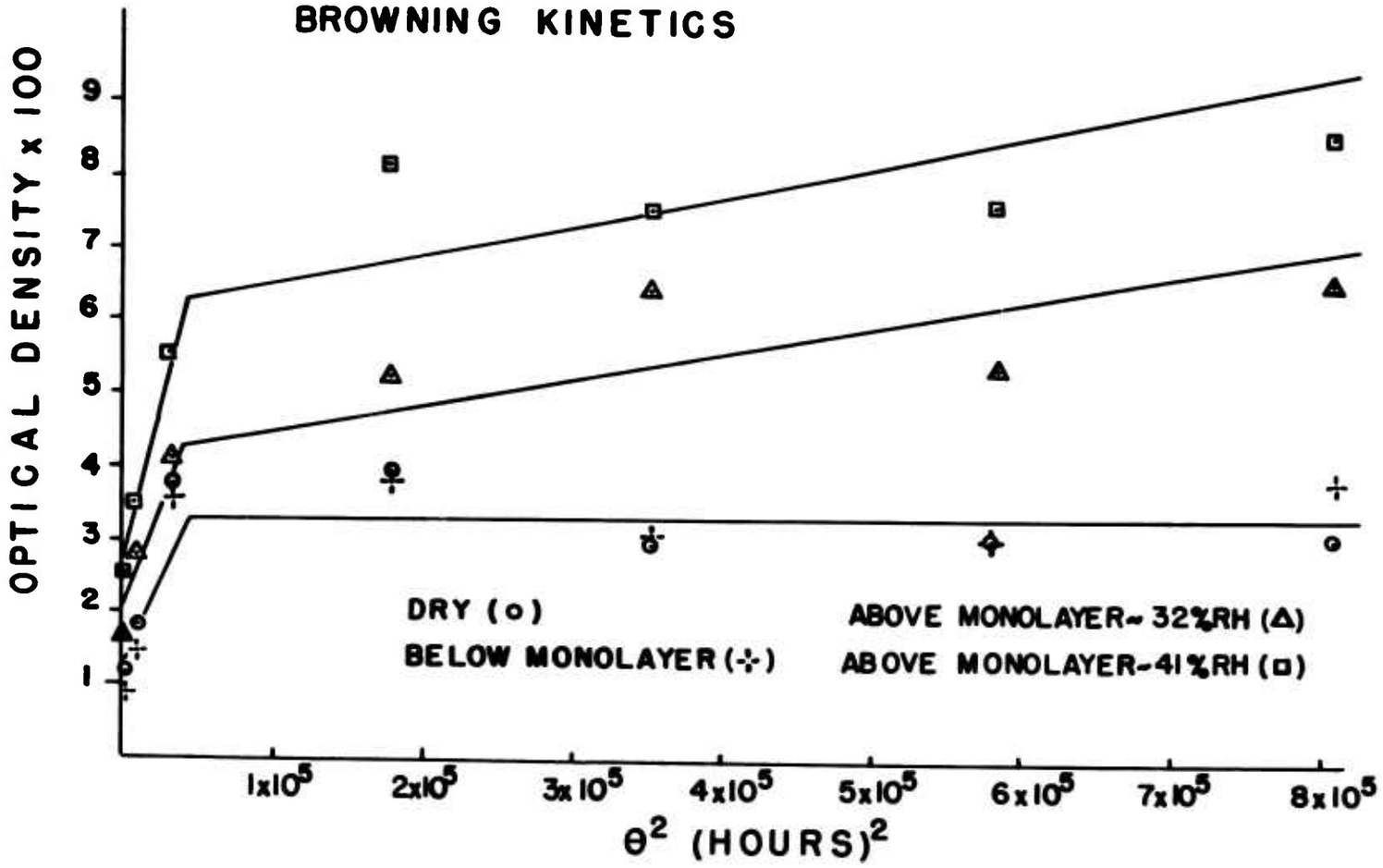
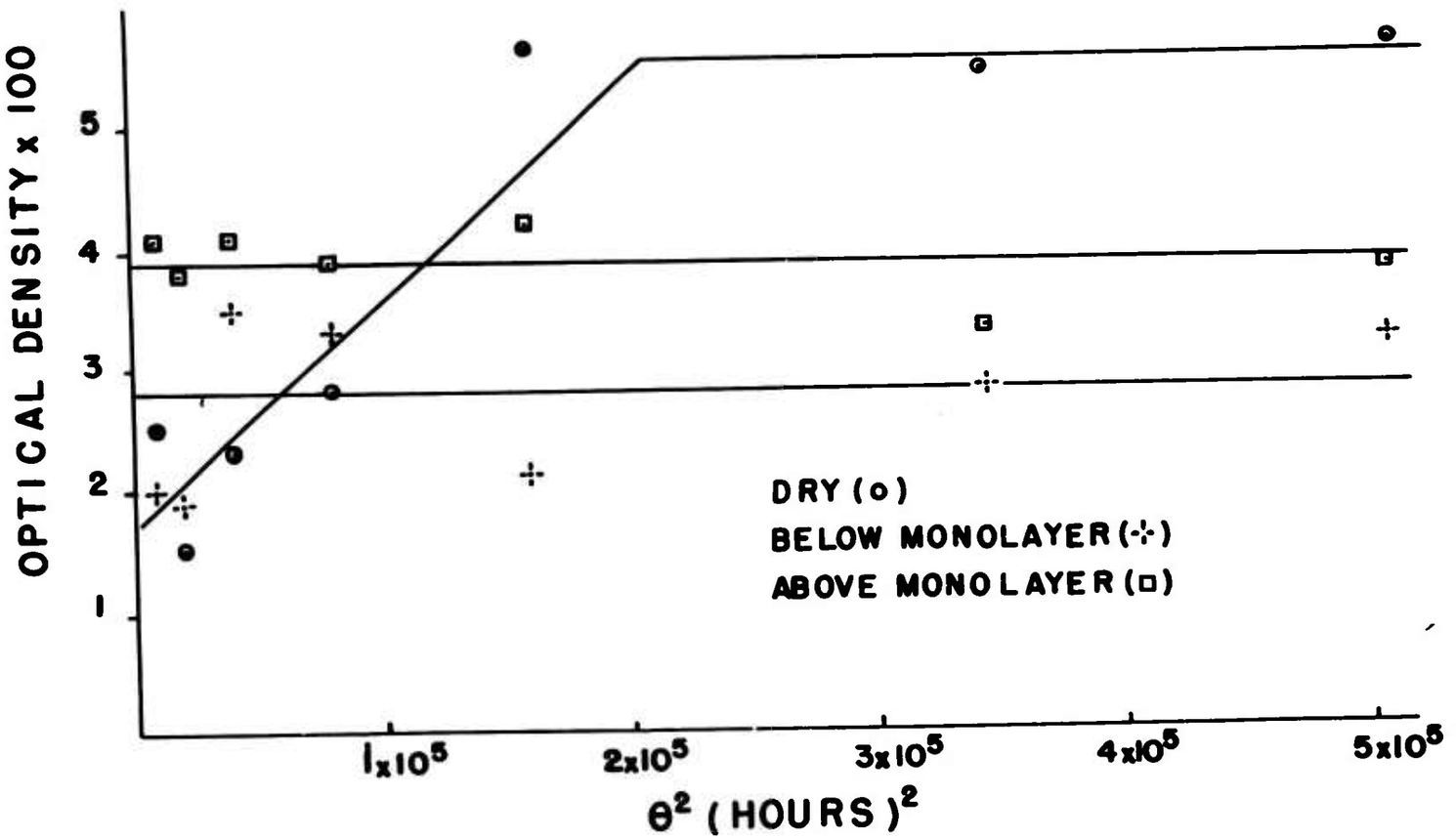


FIGURE 13

FREEZE–DRIED SALMON - RUN N° 2

BROWNING KINETICS



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Theodore P. Labuza

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13. ABSTRACT

Model systems similar in composition to three types of freeze-dried foods were developed and used in studies on the effects of composition on the rates of deteriorative reactions at 55°C. Oxidation of lipids was found to proceed most rapidly at low water contents, while non-enzymatic browning and oxidation of proteins was more rapid at elevated water contents. Antioxidants and chelating agents had only a limited effectiveness which was also dependent on moisture content. During studies on dehydrated systems it was observed that hydrolysis of sucrose can occur even at low water contents and that the reducing sugars produced in the hydrolysis undergo considerable browning. Moisture contents corresponding to monolayer coverage by water were generally optimal in minimizing deteriorative changes.

Studies on actual foods were limited to a 3-week storage study at 55°C. At this relatively high temperature non-enzymatic browning was found to be the cause of deterioration. Under these conditions none of the foods were found to be satisfactory after storage, and oxidative changes were less important than browning. Consequently over-all quality of materials packaged under vacuum showed no improvement over of materials packaged in air. Moisture content did have a significant effect, high water contents resulting in more extensive deterioration.

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space feeding systems stability						
deterioration in freeze dehydrated foods						
space food storage						

FOREWARD

The work described in this report was accomplished at the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts, under contract Number AF41(609)-2981 and Task Number 775803. Dr. Marcus Karel served as Principal Investigator, and Dr. Theodore P. Labuza served as Co-investigator.

The monitoring agency was the Physiology Branch, USAF School of Aerospace Medicine, Aerospace Medical Division (AFSC), Brooks Air Force Base, Texas. The contract monitor was Major Norman D. Heidelbaugh, USAF, VC. Monitor at the close of the contract was Major William T. Ashby, USAF, VC.

This report covers work performed during the period 15 May 1966 to 15 May 1967.